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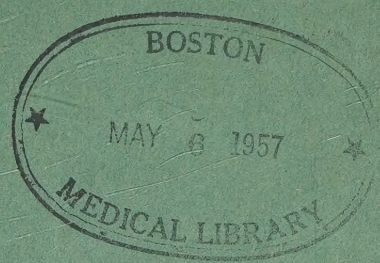
VETERINARY REVIEWS AND ANNOTATIONS



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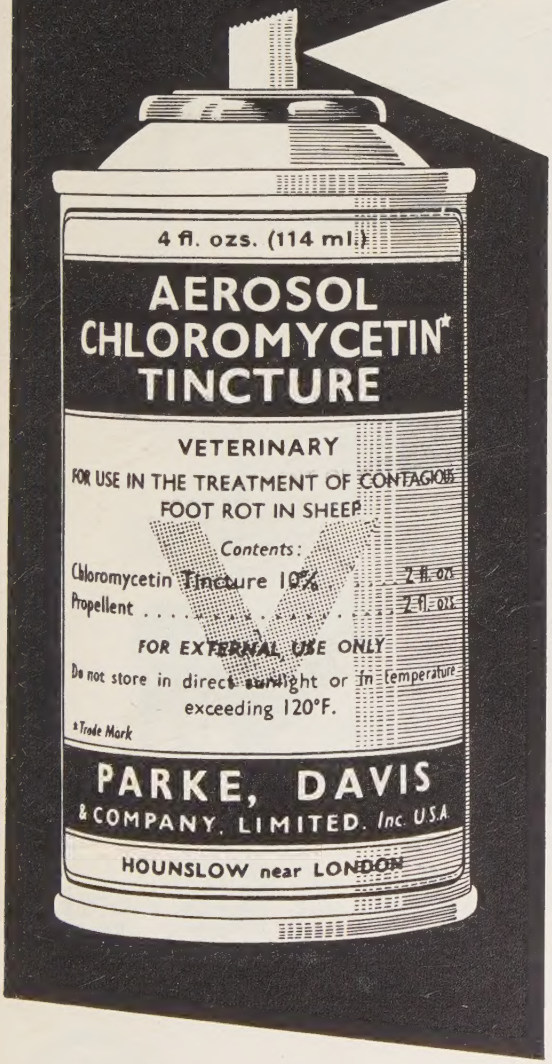

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CORRIGENDUM

Vol. II, part 2. page 81, para. 4. End of line 7 to end of para. should read:- "Venn et al. (1947) have shown that the Fe content per 100 g. body weight of new-born pigs does not vary greatly according to their size and it is to be expected that, under conditions of iron insufficiency, the larger more rapidly growing pigs will be at a disadvantage as compared with the smaller ones and will be more susceptible to the disease."

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 3 **April, 1957** *Part 1*

The Classification of Trypanosomes of Veterinary and Medical Importance

BY

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Wellcome Laboratories of Tropical Medicine, London

INTRODUCTION

The various forms of animal and human trypanosomiasis differ so widely in their host-range, epidemiology, clinical manifestations and response to chemotherapy, that the exact identification of the species of trypanosomes involved — in other words, the parasitological diagnosis of the disease — is a matter of considerable practical importance. Unfortunately, many of the veterinary and medical workers engaged in the field of trypanosomiasis are not familiar with the systematics of trypanosomes. Consequently, in their preoccupation with treatment and reclamation, they tend to lose sight of the trypanosomes themselves, with the result that the aetiology of the infections is often determined solely on clinical or epidemiological grounds, and the collective term "trypanosomiasis" is frequently applied indiscriminately to diseases caused by distinct species of trypanosomes. All this uncertainty could be avoided if the differential characters of the trypanosome species were known to and applied by the workers concerned.

In general, the object of systematics is twofold: one is to provide a practical scheme for the identification and differentiation of species, the other is to arrange the species in groups according to the degree of their relationship or affinities. While in the former case the desired end can be achieved by devising an artificial key, in the latter case the evolutionary history of the species is taken into consideration, and the classification is based on their phylogeny.

These two methods of approach are reflected in the history of the systematics of the mammalian trypanosomes. Thus, the earlier attempts to classify the species, by Laveran & Mesnil (1912) and Yorke & Blacklock (1914), were purely artificial, since they were based on minor structural differences, thereby uniting morphologically distinct forms and separating similar ones. An advance was made by Bruce (1914, 1915), who recognized the affinities of African trypanosomes possessing common characters by grouping them together. Previously Duke (1913) had introduced the mode of development in the insect-vector as an essential criterion for differentiating the tsetse-borne trypanosomes. The classification on these lines was further developed and extended to other mammalian trypanosomes by Knuth & du Toit (1921) and Wenyon (1926). Finally, in a series of publications Hoare (Hoare & Coutelen, 1933; Hoare, 1948, 1949) advocated what he regards as a natural classification, based on phylogenetic considerations. The merit of this classification is that it not only reflects the affinities between various trypanosomes but also brings together systematically related species, which show similarities in their physiology, antigenic constitution and host-parasite relationships. These features, in their turn, have a direct bearing on the response of the trypanosomes to chemotherapy, on the immunological reactions of the host, and on the clinical manifestations of the diseases caused by them.

PHYLOGENETIC RELATIONSHIPS

A phylogenetic classification of the mammalian trypanosomes aims at arranging related groups in accordance with the historical process of their evolution, the course of which is deduced from evidence presented by existing forms. In the flagellate family Trypanosomidae all the genera (including Trypanosoma) are closely related to each other, since their structure and life-cycles illustrate the main phases of evolution through which they must have passed. The arguments supporting this evolution, which have been fully discussed elsewhere (Hoare, 1948), are briefly as follows. Since the stages of development of trypanosomes in the insect-vector closely resemble those of the monogenetic trypanosomid flagellates of insects (genera Herpetomonas, Crithidia), it is thought that trypanosomes have evolved from the parasites of blood-sucking insects. In some mammalian trypanosomes (e.g. Trypanosoma lewisi, T. cruzi) the infective (metacyclic) forms develop in the hind gut ("posterior station") of the vector and are transmitted to new mammalian hosts by faecal contamination. Because the life-cycle of these trypanosomes in the insect-host corresponds to that of the purely insect flagellates, it may be assumed that trypanosomes of this type represent the more primitive and ancient forms. As regards those mammalian trypanosomes (represented by African pathogenic trypanosomes) which complete their development in the salivary medium of the "anterior station" (proboscis or salivary glands) of the vector and are transmitted by inoculation, through its bite, their origin is not so clear, because no comparable development is known among purely insect flagellates. It is probable that this life-cycle was a secondary acquisition in certain trypanosomes which originally completed their development in the hind gut of their vectors. In fact, this transition is

actually taking place in T. rangeli (= T. ariarii), which has a dual life-cycle in the triatomid bug, Rhodnius prolixus: one proceeding in the intestine, with the production of metacyclic trypanosomes in the hind gut, the other involving penetration of the flagellates into the body cavity and invasion of the salivary glands. Transmission of T. rangeli is accordingly effected both by the contaminative and inoculative methods (cf. Groot, 1952; Grewal, 1956b). However, the pathogenic African trypanosomes must have evolved in a different way, for in the tsetse-fly the flagellates migrate directly from the gut to the mouth parts and have never been detected in the body cavity. It is conceivable that they were originally parasites of wild ruminants, which developed in the "posterior station" of some insect-vector and were also transmitted mechanically by tsetse-flies. In the course of time they might have adapted themselves to cyclical development in the "anterior station" of Glossina, which eventually became their new intermediate hosts.

In T. vivax we have an example of the initial phase of this adaptation, when the development of the trypanosomes does not proceed beyond the proboscis of the vector; T. congolense exemplifies the next phase, when the development extended to the midgut as well, while the final phase is reached in T. brucei, which utilizes the salivary glands for the development of the metacyclic forms.

Further evidence of the phylogenetic relationships between mammalian trypanosomes is provided by the difference in the susceptibility of their vectors to infection. Thus, in the case of T. cruzi practically 100% of the bugs become infected, whereas the infectivity of tsetse-borne trypanosomes to Glossina is much lower, being less than 1% in T. brucei. This difference gives an indication of the degree of adaptation established between parasite and vector in the course of evolution. Since in trypanosomes transmitted by inoculation the adaptation is imperfect, they must have evolved later than trypanosomes transmitted by contamination. The imperfect adaptation of tsetse-borne trypanosomes is also reflected in the ease with which they lose the power to develop in the insect-host and revert to mechanical transmission, like T. vivax in South America. A peculiar phase of evolution was reached by T. equiperdum, which is presumably derived from T. evansi by complete emancipation from the insect-vector and adaptation to direct transmission from mammal to mammal.

DIAGNOSTIC CRITERIA

The chief differential characters of mammalian trypanosomes are based on the morphology of the blood forms. Although these are well known and are described in most text-books (e.g. Wenyon, 1926; Hoare, 1949), there are a number of cytological features of diagnostic value to which little attention is paid, such as the size and position of the kinetoplast, which differ in various groups (Hoare, 1938; Schwetz, 1938). Apart from measurements characterizing different species of trypanosomes, statistical methods may be a valuable aid for the separation of related groups and for the study of variation. Thus, the independence of T. uniforme was in doubt until mensural data were obtained for its differential diagnosis from T. vivax (Hoare & Broom, 1938), while, in the case of

trypanosomes of the Brucei group (Fairbairn & Culwick, 1946), T. simiae (Culwick & Fairbairn, 1947) and T. evansi (Hoare, 1956), exact biometrical criteria were provided for their polymorphism. It has also been demonstrated that variations in the virulence of bovine strains of T. vivax (Fairbairn, 1953) and of equine strains of T. evansi (Hoare, 1956) are correlated with differences in the mean lengths of the trypanosomes. However, the application of biometry to the differentiation of intraspecific strains or races may be misleading on account of the wide variation sometimes observed even within populations of the same strain (Hoare, 1956).

Additional clues of taxonomic value are provided by biological characters. Among these, the site and stages of development in the insect-vector serve as a basis for distinguishing the major groups of trypanosomes. As regards the host-parasite relationships, which are manifested chiefly in the virulence and host-specificity of the trypanosomes, they are subject to variation and cannot, therefore, always be relied upon for the diagnosis of trypanosome species. Thus, the inability of T. brucei to infect man is a stable characteristic of this species, but T. rhodesiense apparently represents merely a more virulent variant of T. gambiense. Similarly, T. vivax comprises strains differing in the degree of virulence for cattle. On the other hand, in T. evansi there are distinct hostal strains, specially adapted to camels, horses or cattle (Hoare, 1956). However, in some trypanosomes the host-range and infectivity to new hosts are not rigidly fixed. For example, T. simiae, which was thought to be restricted to pigs, is now known to occur in cattle, horses and camels as well, while the refractoriness of rodents can be overcome, as was shown in the case of T. vivax and T. simiae, which are now successfully maintained in the laboratory (Desowitz & Watson, 1951–53).

In the past much importance was attached to the cross-immunity test as a method for the differentiation of morphologically indistinguishable strains (e.g. in T. evansi), but the recognition of serologically distinct relapse, geographical and hostal strains has discredited this method.

REVISED CLASSIFICATION

The classification of mammalian trypanosomes outlined below is based on that originally proposed by the present writer (Hoare & Coutelen, 1933; Hoare, 1936, 1938, 1948, 1949, 1956). It has now been brought up to date and revised in the light of recent additions to our knowledge, including the discovery of new species and varieties, and the recognition of intraspecific races and strains. The revised classification is condensed in the form of (a) a descriptive key, and (b) a table containing the most important data relating to the species, with (c) illustrations (Figs. 1, 2) of all the species of veterinary and medical interest. This presentation enables the reader to appreciate at a glance the phylogenetic relationships between the trypanosomes, and to make practical use of this scheme for the differential diagnosis of species. A more detailed account of the better known species is given in text-books (Wenyon, 1926; Hoare, 1949; and others), but, in the case of less known species and varieties, explanatory notes are appended.

KEY TO MAMMALIAN TRYPANOSOMES OF VETERINARY AND MEDICAL INTEREST

Genus Trypanosoma:

Flagellates of the family Trypanosomidae which pass through leishmanial, crithidial and trypanosome stages in the vertebrate and invertebrate hosts.

SECTION A

Morphology in Mammal:

Kinetoplast not terminal, large. Free flagellum always present. Posterior end of body pointed. Division in crithidial, leishmanial or trypanosome stages.

Biology:

Multiplication in mammal typically discontinuous. Development of metacyclic trypanosomes in hind gut (posterior station) of vector (in T. rangeli also in salivary glands: anterior station). Transmission contaminative through faeces (in T. rangeli also inoculative). Trypanosomes slightly or not pathogenic.

I. LEWISI GROUP (Fig. 1)

(a) Reproduction unknown: T. melophagium (Fig. 1, a).

(b) Reproduction by binary fission in crithidial stage: T. theileri (Fig. 1, b, c).

(c) Reproduction by multiple fission in crithidial stage: T. lewisi (Fig. 1, d, e), T. duttoni (Fig. 1, f, g).

(d) Reproduction by binary fission in leishmanial stage: T. cruzi (Fig. 1, h, i).

(e) Reproduction by multiple fission in leishmanial stage: T. nabiasi (Fig. 1, j, k).

(f) Reproduction by binary fission in trypanosome stage: T. rangeli (= T. ariarii) (Fig. 1, l, m).

SECTION B

Morphology in Mammal:

Kinetoplast terminal or subterminal. Posterior end of body blunt. Division in trypanosome stage.

Biology:

Multiplication in mammal continuous. Development of metacyclic trypanosomes in proboscis or salivary glands (anterior station) of vector (except Evansi subgroup). Transmission inoculative, through bite (except T. equiperdum). Trypanosomes pathogenic.

II. VIVAX GROUP (Fig. 2, A)

Monomorphic forms. Posterior end of body typically rounded. Free flagellum always present. Kinetoplast large, terminal. Undulating membrane inconspicuous. Development in Glossina: proboscis only.

(a) Long forms: T. vivax (Fig. 2, a, b).

(b) Short forms: T. uniforme (Fig. 2, c, d).

III. CONGOLENSE GROUP (Fig. 2, B)

Monomorphic or polymorphic forms. Free flagellum absent or present. Kinetoplast medium, marginal. Development in Glossina: midgut and proboscis.

(a) Monomorphic (short forms, free flagellum absent or short, undulating membrane inconspicuous): T. congolense (Fig. 2, e).

(b) Dimorphic (short and long forms, free flagellum absent or short, undulating membrane inconspicuous): T. dimorphon (Fig. 2, e, f).

(c) Polymorphic (short forms without free flagellum; and long forms, comprising stout ones with conspicuous undulating membrane and slender ones with inconspicuous undulating membrane, free flagellum absent or present): T. simiae (Fig. 2, e–i).

IV. BRUCEI GROUP (Fig. 2, C)

Monomorphic or polymorphic forms. Free flagellum present or absent. Kinetoplast small, subterminal (absent in T. equinum). Undulating membrane conspicuous. Development in Glossina: midgut and salivary glands (except Evansi subgroup).

(a) Monomorphic (stout forms with short free flagellum):

1. Suis subgroup: T. suis (Fig. 2, j, k).

(b) Polymorphic (slender, intermediate and stumpy forms):

2. Brucei subgroup — polymorphism constant (stumpy forms always present): T. brucei, T. rhodesiense, T. gambiense (Fig. 2, l, m, n).

3. Evansi subgroup — polymorphism inconstant (stumpy forms rare or sporadic); intermediate host absent:

   (i) Transmission mechanical by insects: T. evansi (= T. hippicum, T. venezuelense, etc.) (Fig. 2, 1, m), T. equinum.

   (ii) Transmission by contact (coitus) from mammal to mammal (equines): T. equiperdum (Fig. 2, 1, m).

Explanatory notes to key

(i) The reproduction of T. theileri was first described by Reichenow (1940), that of T. nabiasi by Grewal (1956a).

(ii) Although morphologically T. rangeli belongs to the Lewisi group, its dual life-cycle in the insect-vector entitles it to a position between Sections A and B, as a transitional form.

(iii) The name T. dimorphon, created by Laveran & Mesnil in 1904, was frequently misused and had gradually passed out of circulation, so that at present few workers recognize the independence of this species. Nevertheless, there can be no doubt about the existence of dimorphic strains conforming to the original T. dimorphon (Schwetz, 1938; and see iv below).

(iv) The following new varieties have recently been described from pigs in the Belgian Congo: T. congolense var. urundiense, T. c. var. berghei and T. c. var. mossoense (Peel & Chardome, 1954b, c; Chardome & Peel, 1954). In a critical analysis of the work of these authors, based on the original preparations, Hoare (in press) has shown that the first two varieties are indistinguishable and correspond to T. dimorphon (see iii above), whereas the third conforms to T. simiae.

(v) The most important addition to African trypanosomes was the re-discovery in the Belgian Congo (by Peel & Chardome, 1954a) of T. suis Ochmann, 1905, which proved to be an independent species pathogenic to pigs. Since its development in Glossina terminates in the salivary glands, it is referred to the Brucei group as a monomorphic subgroup (Hoare, 1956).

(vi) The demonstration of polymorphism in T. evansi (Hoare, 1956) has confirmed its affinity to and position in the Brucei group.

(vii) Except for the absence of a kinetoplast in T. equinum, this species can be illustrated by Fig. 2, 1, m.

CHIEF CHARACTERISTICS OF TRYPANOSOMES OF VETERINARY AND MEDICAL INTEREST

Species of *Trypanosoma*	Length (micra)	Mammalian hosts	Susceptible laboratory animals	Insect hosts or vectors	Geographical distribution	Effect on host
I. *LEWISI* GROUP						
1. *T. melophagium* Flu, 1908 (Fig. 1, a)	50-60	Sheep	None	Sheep ked	Cosmopolitan	Non-pathogenic
2. *T. theileri* Laveran, 1902 (Fig. 1, b)	60-70	Bovines	None	Tabanid flies	Cosmopolitan	Non-pathogenic
3. *T. lewisi* (Kent, 1880) (Fig. 1, d)	26-34	Rats	None	Fleas	Cosmopolitan	Non-pathogenic
4. *T. duttoni* Thiroux, 1900 (Fig. 1, f)	28-34	Mice	None	Fleas	Cosmopolitan	Non-pathogenic
5. *T. cruzi* Chagas, 1909 (Fig. 1, h)	16-20	Man, dogs, cats, armadillos, opossums	Rodents	Triatomid bugs	America	Pathogenic: Chagas' disease
6. *T. nabiasi* Railliet, 1895 (Fig. 1, j)	21-30	Rabbits	None	Fleas	Cosmopolitan	Non-pathogenic
7. *T. rangeli* Tejera, 1920 (= *T. ariarii*) (Fig. 1, l)	26-36	Man, dogs, opossums, monkeys	Mice, rats	Triatomid bugs	Central and South America	Non-pathogenic
II. *VIVAX* GROUP						
8. *T. vivax* Ziemann, 1905 (Fig. 2, a, b)	20-26	Bovines, sheep, goats, antelopes	Rodents	Tsetse flies (*Glossina*)	Tropical Africa	Pathogenic: Souma
9. *T. uniforme* Bruce *et al.*, 1911 (Fig. 2, c, d)	12-20	Bovines, sheep goats, antelopes	None	Tsetse flies	Tropical Africa	Pathogenic

III. CONGOLENSE GROUP						
10. T. congolense Broden, 1904 (Fig. 2, e)	9-18	Bovines, equines, pigs, sheep, goats, dogs	Rodents	Tsetse flies	Tropical Africa	Pathogenic
11. T. dimorphon Laveran & Mesnil, 1904 (Fig. 2, e, f)	12-25	Horses, pigs, bovines	Rodents	Tsetse flies	Tropical Africa	Pathogenic
12. T. simiae Bruce et al., 1911 (Fig. 2, e–i)	12-24	Pigs, camels, bovines, horses, wart-hogs	Rabbits	Tsetse flies	Tropical Africa	Pathogenic
IV. BRUCEI GROUP						
13. T. suis Ochmann, 1905 (Fig. 2, j, k)	14-19	Pigs	None	Tsetse flies	Belgian Congo, Tanganyika	Pathogenic
14. T. brucei Plimmer & Bradford, 1899 (Fig. 2, l, m, n)	12-35	All domestic animals, antelopes	Rodents etc.	Tsetse flies	Tropical Africa	Pathogenic: Nagana
15. T. rhodesiense Stephens & Fantham, 1910 (Fig. 2, l, m, n)	12-35	Man, antelopes	Rodents etc.	Tsetse flies	Tropical East Africa	Pathogenic: Rhodesian sleeping sickness
16. T. gambiense Dutton, 1902 (Fig. 2, l, m, n)	12-35	Man	Rodents etc.	Tsetse flies	Tropical Africa	Pathogenic: Gambian sleeping sickness
17. T. evansi (Steel, 1885) (= T. hippicum, T. venezuelense, etc.) (Fig. 2, l, m)	15-34	Bovines, camels, equines, dogs, etc.	Rodents	Tabanid flies	Cosmopolitan (tropical and subtropical)	Pathogenic: Surra etc. in Old World; Derrengadera, Murrina in New World
18. T. equinum Voges, 1901	22-24	Equines	Rodents	Tabanid flies	South America	Pathogenic: Mal de Caderas
19. T. equiperdum Doflein, 1901 (Fig. 2, l, m)	25-28	Equines	Rabbits	No vectors (transmission by coitus)	Southern Europe, North Africa, Asia	Pathogenic: Dourine

Intraspecific groups

It is now necessary to consider the systematic position of strains of trypanosomes which are morphologically indistinguishable, but differ in their host-parasite relations, such as host-specificity (e.g. T. evansi, T. brucei – T. rhodesiense – T. gambiense) and type of disease produced by them (e.g. T. evansi – T. equiperdum). To these may be added the antigenically distinct relapse strains among pathogenic trypanosomes. All such intraspecific strains represent "biological races", which can be classified in different taxa, according to the degree of divergence and stability of the biological characters distinguishing them (Hoare, 1955). From this point of view, the retention of specific status is justified for T. evansi and T. equiperdum, whereas members of the Brucei subgroup can be regarded as subspecies: T. brucei brucei, T. brucei gambiense and T. brucei rhodesiense. As regards strains differing in minor clinical, serological or hostal features, they represent demes, which correspond to "types" among bacteria and are combined with a prefix to denote the nature of the differential criterion, e.g. T. evansi with cameline, equine and bovine xenodemes or hostal races. Finally, relapse strains, which represent environmentally induced transient populations, correspond to morpha, a term used to denote enduring modifications.

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Explanation of Figures

Fig. 1. Trypanosomes of the Lewisi group (x 2000).

(The first member in each pair represents an adult form, the second a dividing one).

a. T. melophagium; b, c. T. theileri; d, e. T. lewisi; f, g. T. duttoni; h, i. T. cruzi; j, k. T. nabiasi; l, m. T. rangeli (= T. ariarii).

(Adapted from various authors).

Fig. 2. Pathogenic trypanosomes (x 2000).

A. Vivax group: a, b. T. vivax; c, d. T. uniforme;
B. Congolense group: e. T. congolense; e, f. T. dimorphon; e–i. T. simiae;
C. Brucei group: j, k. T. suis; l, m, n. T. brucei, T. rhodesiense, T. gambiense; l, m (± n). T. evansi (= T. hippicum, T. venezuelense), T. equinum (but without kinetoplast), T. equiperdum.

(Original).

Fig. 1.

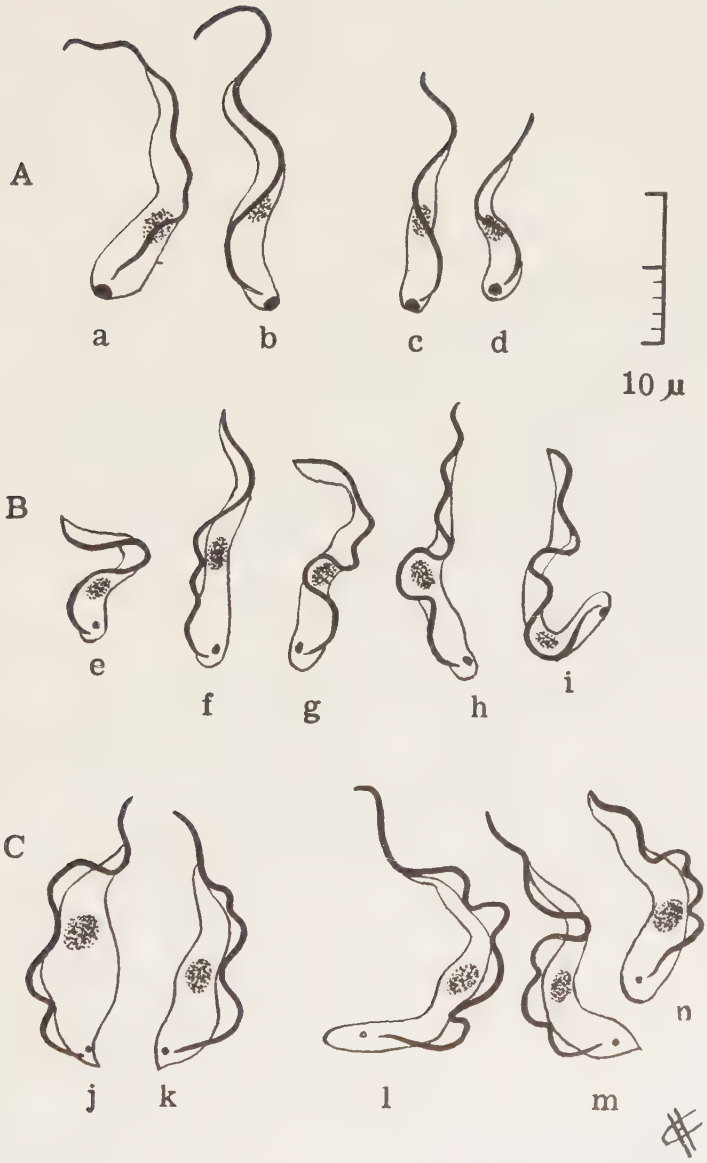

Fig. 2.

The Chemotherapy of Animal Trypanosomiasis with particular reference to the Trypanosomal Diseases of Domestic Animals in Africa

BY

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INTRODUCTION

Trypanosomes have a notable place in the history of chemotherapy. They featured in the early experimental work on synthetic compounds that was just beginning in the laboratories of Europe at the beginning of this century, and there is reason to suppose that if Thomas, working in Runcorn (Liverpool) in 1904, had not demonstrated the curative action of atoxyl on Trypanosoma gambiense in mice, Ehrlich would not have been led to 606 (Salvarsan) for syphilis. Thereafter, as experimental chemotherapy gathered momentum under the great stimulus of Ehrlich, trypanosomes became part, as it were, of the laboratories concerned, and they lent their name to some of the early and now historical substances such as Trypan red (1904), Trypan blue (1904) and Trypasafrol (1912).

At first, it was the trypanosomes of man, T. rhodesiense and T. gambiense, that attracted attention, and one of the early great successes of the new chemotherapy was Bayer 205 (Germanin, Naganol, Suramin, Antrypol etc.) announced for human trypanosomiasis in 1920, and found to be of use, too, against T. brucei, T. equinum, T. equiperdum, and T. evansi. The trypanosomal diseases of cattle in Africa, undoubtedly now the most important of all the diseases caused by trypanosomes, waited until the 'Thirties before some attention was focused on them. In Africa itself tartar emetic was being used for treatment, but in Germany and in England the search for other drugs against T. congolense was being pursued.

The search was more successful than the immediate results of the original investigations indicated, for all the most modern drugs for animal trypanosomiasis are related to the substances that came to light in the 'Thirties. Ethidium is in a direct line of descent from phenanthridinium 897 (Phenidium) synthesized by Morgan & Walls in 1938, and shown to be active against T. congolense in mice by Browning in Glasgow in the same year; the discovery of Antrycide owes much to the work of Iensch (1937) that led to Surfen C, and Berenil goes back to the work on the diamidines done by Ewins and his collaborators at Dagenham, by Yorke and his colleagues in Liverpool, by King in the Medical Research Council laboratories in London, and before that to the demonstration of trypanocidal properties in Synthalin (decamethylene-diguanidine hydrochloride) (von Jansco & von Jansco, 1936).

This review will be concerned in detail only with the modern drugs and it is to gain perspective and to pay homage that a very brief history has been given. For a fuller account of the early work the reader is

referred to Findlay (1950).

The arrangement of the review needs some comment. I have thought it best to deal with the drugs and their properties first, then very briefly (and consequently, in some measure, dogmatically) with the treatment of the individual diseases, and finally to say something of the problems that remain. The aspects discussed are essentially practical, and for an introduction to the literature concerning the mode of action of the trypanocidal drugs — about which we know very little that helps us to discover others — reference should be made to the reviews by Work & Work (1948), Findlay (1950), von Brand (1951), and Sexton (1953), and the papers by Hawking et al. (1937), Hawking (1944), Ormerod (1951a, b), Sen et al. (1954), Hawking & Thurston (1955) etc.

THE DRUGS IN USE AGAINST THE TRYPANOSOMAL DISEASES OF DOMESTIC ANIMALS

Tartar emetic, antimosan and Surfen C are now hardly, if ever, used; and the cinnoline 528 of Lourie et al. (1951) was found wanting. Suramin is still widely used for the treatment of T. evansi in camels, but its attributes and its defects are now well known and need not be discussed here. We are left with Berenil, the phenanthridinium compounds, Antrycide and certain "complexes" of suramin with these drugs. Other substances have been mentioned in recent papers as active against trypanosomes in small laboratory animals, but no information is yet available concerning their activity in the domestic animals, and so, for reasons of space, they have not been considered.

Berenil *

This substance has the constitution:

Its properties have been described by Fussgänger (1955), and Bauer (1955a, b). It has a relatively poor action in mice against trypanosomes of the Brucei group, and a more marked action against T. congolense. The minimum curative dose for the last species is given as 1–2.5 mg./kg. intramuscularly in the dog, and 2.5 mg./kg. subcutaneously in the mouse (in my own experiments more than 5 mg./kg. is necessary to cure the Busimbi, Uganda strain of T. congolense in mice).

Reports of experiments with the drug in cattle are few. Bauer (1955a) described an experiment in Western Tanganyika in which three groups of cattle, six beasts in each group, all infected with T. vivax, were treated respectively with 1, 2, or 3 mg./kg. intramuscularly. As a result of the experiment the standard dose of 3 mg./kg. intramuscularly was

* marketed by Hoechst of Germany.

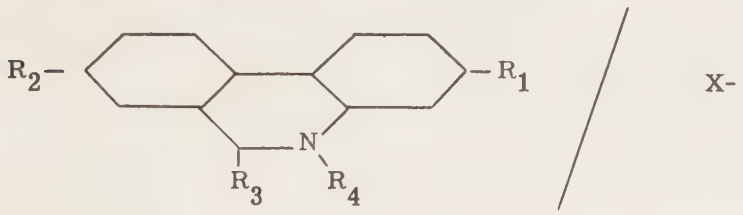
decided upon. This dose was used in Portuguese East Africa to treat 500 cattle, the stock of 12 European farmers. Between 10 and 60% of each herd carried natural infections of T. vivax with or without T. congolense and T. brucei. Trypanosomes were said to have disappeared within 24 to 48 hours, and there were no side effects from the treatment. Subsequent examinations appear to have been limited, but at least four possible relapses with T. congolense or T. vivax were found amongst 26 animals followed up for six months. In a second paper (1955b) Bauer stated that some 800 cattle had been treated for trypanosomiasis with Berenil; 2 or 3 mg./kg. intramuscularly had always given cures, and 1 mg./kg. had been sufficient to cure T. congolense.

Milne et al. (1955) reported an experiment from Tanganyika in which 20 cattle infected with T. congolense were treated with 2 mg./kg., five subcutaneously and 15 intramuscularly. Trypanosomes disappeared from the blood within 5 to 40 hours, and the cattle remained apparently free from infection for 150 days. Re-infection was achieved in all except one animal.

Berenil has only a poor action against T. simiae (Bauer, 1955a).

The Phenanthridinium Compounds. I.

These have the general formula:



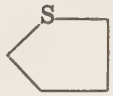
Activity in this type of compound against T. congolense was discovered by Browning et al. (1938) whose work extended over many years and which culminated in 1943 with the compound 1553 that came to be known as Dimidium bromide. Further studies of the type, particularly since the war, were then made in the laboratories of Burroughs Wellcome and Co. and Boots Pure Drug Co.

Of the very considerable number of compounds tested in the laboratory nine have been tried against infections in cattle, and of these, six were discarded in their early experimental stages for one reason or another (see, e.g. Goodwin & Unsworth, 1952; Goodwin & Chandler, 1952; Burdin & Plowright, 1952a, b; Burdin, 1953; Wilde & Robson, 1953; Neal & Karib, 1954).

The discarded compounds are:

B. W. & Co. 150. C. 47 ($R_1R_2 = NH_2$; R_3 = NH_2; $R_4 = CH_3$; X = Cl)

B. W. & Co. 676. C. 47 ($R_1R_2 = NH_2$; $R_3 =$ NO_2; $R_4 = CH_3$; $X = Cl$)

B. W. & Co. 621. C. 47 ($R_1R_2 = NH_2$; $R_3 =$  ; $R_4 = CH_3$; $X = Br$)

B. W. & Co. 196. C. 48 ($R_1 = NH_2$; $R_2 = C_2H_5O$; $R_3 =$ NH_2; $R_4 = CH_3$; $X = Cl$)

Boots RD 1427 ($R_1R_2 = NH_2$; $R_3 =$; $R_4 = C_3H_7$; $X = Br$)

Boots RD 1446 ($R_1R_2 = NH_2$; $R_3 =$; $R_4 = CH_2.CH.CH_2$; $X = Br$)

The three that progressed and came to be used more widely are:

Phenidium chloride or phenanthridinium 897 ($R_1 = H$; $R_2 = NH_2$; $R_3 =$ NH_2; $R_4 = CH_3$; $X = Cl$)

Dimidium bromide or phenanthridinium 1553 ($R_1R_2 = NH_2$; $R_3 =$; $R_4 = CH_3$; $X = Br$)

Ethidium bromide or Boots RD 1572 ($R_1R_2 = NH_2$; $R_3 =$; $R_4 = C_2H_5$; $X = Br$)

Phenidium chloride

This was one of the two substances described when the phenanthridinium compounds were first announced as "a new type of trypanocidal agent" (Browning et al., 1938). It was tried against T. congolense in cattle in Africa by Hornby et al. (1943) and by Carmichael & Bell (1944a). In the experiments of Hornby and his colleagues the only animal

treated with 4 mg./kg. subcutaneously relapsed; it also had a large swelling at the site of injection which persisted for the two months before it was killed. Of 20 given 2 mg./kg. intramuscularly 14 were cured, one was doubtfully cured and five relapsed. The intramuscular injection led to some local reaction and some slight lameness. Carmichael & Bell were led to prefer intravenous treatment, partly because they thought it more efficacious, partly because of the local reactions following intramuscular treatment. Doses greater than 3 mg./kg. intravenously were dangerous and 2 mg./kg. cured only about half the animals.

Phenidium chloride has very obvious drawbacks to modern eyes, and so it is not without interest to recall that 12 years ago Carmichael & Bell (1944a) could conclude that the drug "must ... be given a high place among the trypanocides tried against this very intractable infection."

Phenidium had a relatively short life. There is a curious lag between the time of its announcement in England (1938) and its trial in Africa (1943) and because of this Dimidium bromide, not discovered until 1943, appears to follow hard on its heels into Africa.

Dimidium bromide

Dimidium bromide was first tried in Uganda against T. congolense (Carmichael & Bell, 1944b; Bell, 1945), and the results were most promising. They indicated that the new drug had a relatively wide margin of safety, that a dose of 1 mg./kg. subcutaneously was sufficient to cure T. congolense, and that injection of the drug by this route caused relatively little irritation whilst intramuscular injection produced a painful swelling and led to marked lameness. The initial field trials with the drug in Uganda in 1945 proved these conclusions to be far too optimistic. In the first, of 187 cattle treated with 2 mg./kg. subcutaneously "nearly all developed symptoms of photosensitization some six weeks after administration of the drug, and 41 subsequently died. In addition, severe local reactions at the site of injection were of frequent occurrence, resulting in sloughing of areas of skin up to 12 inches or more in diameter." (Randall & Beveridge, 1946). Because of these mishaps the dose was reduced to 1 mg./kg., and the injections made intramuscularly in the next trial. No toxic effects were observed and it was estimated that, using this dose, the relapse rate with T. congolense infections probably did not exceed 5% (Randall & Beveridge, 1946).

Further experimental work confirmed in general the conclusions drawn from the use of Dimidium in the field in Uganda, (Barnett, 1946; Randall & Laws, 1947; Randall & Beveridge, 1947; Wilson, 1948a, b; Wilde, 1949). All workers were agreed that so-called photosensitization was a serious hazard in the use of the drug; that 1 mg./kg. was about the highest reasonably safe dose; that the intramuscular route was probably to be preferred because of the sometimes severe local reaction after subcutaneous administration; and that 1 mg./kg. intramuscularly cured the majority but not all cases of T. congolense.

The standard treatment with Dimidium bromide, then, came to be 1 mg./kg. intramuscularly, but some territories, e.g. Kenya and Southern Rhodesia, used doses up to 1.5 mg./kg., and in Southern Rhodesia the intravenous route was preferred even though it led to the veins becoming

partly occluded. Delayed toxicity has always been a fear with those who used the drug, understandably in view of its potential severity (see, e.g. Evans, 1948; Thorold & Plowright, 1952) but nevertheless Dimidium has been of very considerable use in Africa as the annual reports of the Veterinary Departments of such countries as the Republic of the Sudan, Kenya, Nigeria, the Rhodesias etc. emphasize again and again.

One point will have struck the reader, and that is how little attention was given to T. vivax in the experimental studies done with Dimidium. Everyone knew that T. vivax was important, but few attempted to work with it (in later trials with Ethidium and Antrycide — see below — T. vivax was not neglected). The accumulated results from the field, however, finally served to show that this species appeared to be as susceptible as T. congolense and there are a few experiments, e.g. Mornet & Mahou (1949) to confirm this. For the rest, the prophylactic action of Dimidium is very little (annual report of the Veterinary Department, Nigeria, 1947, p. 20) and its action against T. simiae is poor (Wilson, 1948a). The toxicity of the drug is discussed further below.

Ethidium *

Ethidium bromide was announced in 1952 by Watkins & Woolfe who claimed that in mice it was somewhat less toxic than Dimidium bromide, and 10 times as active against T. congolense, 20 times as active against T. brucei, 50 times as active against T. gambiense, and 11 times as active against T. rhodesiense. The reader will have noticed that the chemical difference between Dimidium bromide and Ethidium bromide is slight, merely the difference that in one the quaternizing group is methyl (CH_3) and in the other is ethyl (C_2H_5). The difference in biological properties is therefore remarkable; it is equally remarkable that 14 years went by before the attributes of the ethyl quaternization group were discovered.

Experiments have been done in various parts of Africa with Ethidium bromide, and Table I is an attempt to summarize the therapeutic results achieved.

In the actual experiments summarized in Table I various doses extending over quite a wide range were tried, of course, and on the basis of all the work the manufacturers recommend 1 mg./kg. for routine use which clearly allows latitude with some strains. It is advised, however, that the dose be given intramuscularly, because all the workers who gave it subcutaneously describe the development of a sometimes large swelling at the site of injection with occasional necrosis of the skin. Also, an alternative salt is offered. The initial work was done with the bromide which requires boiling water to bring it into solution whereas the chloride (called Ethidium "C") is soluble in cold water. Like Dimidium bromide, Ethidium is relatively rapid in action, and with effective treatment trypanosomes disappear from the blood within 48 hours.

The promise of Ethidium so apparent in the experimental work is not reflected in a recent report from the Belgian Congo where Thienpont &

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* marketed by Boots Pure Drug Co., England

TABLE I

Action of Ethidium bromide against T. congolense and T. vivax in cattle

Territory	Species	Route	Probable Minimum Curative Dose * mg./kg.	No. of cattle on which result is based	Reference
Sudan	T. vivax **	s/c	0.25	6	Ford et al. (1953a)
Sudan	T. congolense	s/c	0.5–1	7 at 0.5 6 at 1	Ford et al. (1953b)
Kenya	T. congolense	s/c	0.5	3	Wilson & Fairclough (1953)
Tanganyika	T. congolense	s/c	0.3–0.9 (?)+	8 at 0.3 8 at 0.9	Wilde & Robson (1953)
Nigeria	T. vivax (Syringe-passaged)	s/c	1 (Expt. I) 0.1 (Expt. II)	5 2	Unsworth (1954a)
Nigeria	T. vivax (fly-passaged)	s/c	0.5–1	7 at 0.5 9 at 1	Unsworth (1954b)

* Where two doses are quoted a relapse recurred at the lower.
** A debilitating but not a virulent strain.
\+ Of a group of 8 bovines treated with 0.3 mg./kg. 7 were cured and 1 relapsed; an identical result was achieved with 0.9 mg./kg. and possibly a mistake was made or a re-infection occurred.

Herin (1955), working in Astrida, state that 8 relapses occurred amongst 47 cattle infected with T. vivax which were treated with 1 mg./kg. subcutaneously of the bromide.

The Toxicity of the Phenanthridinium Compounds

The delayed toxicity which sometimes follows the injection of Dimidium bromide, the symptoms of which so closely resemble photosensitization, has now been reported from most parts of Africa (see, e.g. Randall & Beveridge, 1947; Stewart, 1947; Evans, 1948; Wilde, 1949; Thorold & Plowright, 1952). At first it was thought to be associated with high doses (2 mg./kg.) but more extended use of the drug showed that it could follow the standard treatment with 1 mg./kg. The general picture is always the same. About six weeks after treatment a proportion of the treated cattle begin to lose condition and may deteriorate rapidly, exhibiting lachrymation and skin lesions. An early symptom may be hyperaemia of the subepithelial tissues of the muzzle and droplets of plasma may be exuded (Plowright et al., 1952). Animals that are mildly affected show "signs of sensitivity and irritation of the muzzle and of the skin of the face and ears, together with slight lachrymation" (Thorold & Plowright, 1952). Animals more severely affected may exhibit "intense irritation and hypersensitivity of the skin — usually of non-pigmented parts exposed to direct sunlight — accompanied by oedematous swellings of the muzzle, face, backs of the ears, base of the horns and dewlap, followed by serous exudation with hardening and cracking of the skin over these areas." Strips of "boarded skin" may peel off the back, flanks and udder exposing a raw granulating surface. The teats may become necrotic and drop off, and the ears may be left raw and misshapen. When the skin over the back and flanks finally hardens the gait is affected and the animals walk stiffly (Thorold & Plowright, 1952).

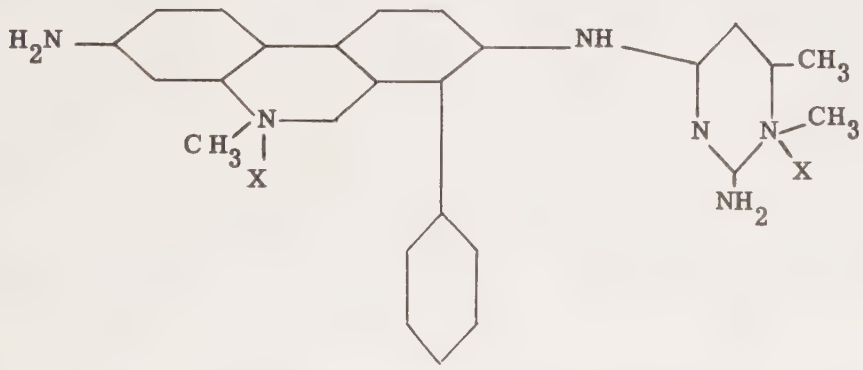
The fact that Dimidium may produce this alarming effect seems undoubtedly due to the toxic action of the drug on the liver (Bell, 1947; Wilde, 1949; Plowright et al., 1952; Burdin & Plowright, 1952a, b), but what the factors are which may exacerbate the liver lesions and why the lesions may lead to the syndrome of photosensitization remain unknown. Delayed toxicity is not an invariable accompaniment of the use of Dimidium bromide at 1 mg./kg., and no one has yet been able to pinpoint the all-important factor linking the sporadic occurrences. It has occurred at high altitudes in Kenya and at low altitudes in the Gold Coast and the Republic of the Sudan; it has been associated with poor grazing in the Republic of the Sudan and in the Gold Coast (Evans, 1948; Stewart, 1947), and with good grazing in Kenya (Thorold & Plowright, 1952) and it has been seen in zebus, mixed zebu and grade crosses, low grade and high grade cattle. Evans thought that cattle in good condition were the most severely troubled, whilst Thorold & Plowright pointed out that the only high grade cattle to be severely affected in the outbreak they witnessed was a Friesian herd in poor condition after an outbreak of foot and mouth disease.

A comparative study of the toxicity of the phenanthridinium compounds, with particular reference to their effect on the liver, has been done at the Kenya Veterinary Laboratory in Kabete (Plowright et al., 1952;

Burdin & Plowright, 1952a, b; Burdin et al., 1952; Burdin, 1953). The liver lesion produced by Dimidium is characteristic and takes the form of a periportal fatty infiltration together with the accumulation of a golden brown pigment, probably bilirubin, in the hepatic and Kupffer cells. It is associated with a loss in weight and condition, a positive direct van den Bergh reaction, a clinically observable jaundice in some cases and an elevated plasma level of alkaline phosphatase. The liver lesion is produced sometimes by 1 mg./kg. and regularly at 2 mg./kg. or more, but symptoms of photosensitization are not invariably present. A similar lesion is obtained with some related compounds (e.g. 150. C. 47), but not with Phenidium at doses up to 8 mg./kg. subcutaneously and 7 mg./kg. intravenously, and not with Ethidium at doses up to 4 mg./kg. The absence of any apparent effect on the liver by Ethidium at this relatively high dose has led to the hope that "photosensitization" will not be a problem bedevilling its use. Confirmation that Ethidium is less toxic for cattle than Dimidium is given by Unsworth (1954a), Ford et al. (1954) and Wilson (1954). Unsworth (1954a) noticed an unexplained rise in temperature (2°–3.6° F.) in a proportion of his uninfected cattle 24–96 hours after they were injected with Ethidium.

The Phenanthridinium Compounds. II.

In a preliminary communication Watkins & Woolfe (1956) described a substance referred to as R.D. 2801 (now called Prothidium*) which is built on the Antrycide plan (see below), but which has a phenanthridinium nucleus in place of the quinoline nucleus of Antrycide linked to the pyrimidine moiety. It has the constitution:



Considerable prophylactic properties are claimed for it, (2 mg./kg. has given protection for approximately six months — personal communication from Dr. G. Woolfe) but trials are still in progress and details are not available.

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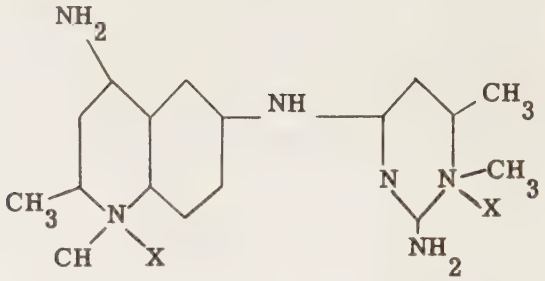
* Boots Pure Drug Co. Ltd.

Antrycide *

This drug was described by Curd & Davey (1949, 1950) and has the constitution:



(X = Cl, Antrycide chloride, or CH_3SO_4, Antrycide methylsulphate)

It has a wider range of action against the various species of trypanosomes than most drugs and is active against the Brucei group, T. congolense, T. equinum, T. equiperdum, T. evansi, T. simiae and T. vivax, but most active against T. congolense. Apart from the intrinsic value of the substance itself its discovery has emphasized two important facts. First, the early experiments were done with two salts, the readily soluble methylsulphate and the sparingly soluble chloride. After subcutaneous or intramuscular injection the former is quickly absorbed whilst the latter is held at the site of injection and only slowly seeps into the transport systems of the body. It was thought, and shown to be true, that the methylsulphate would be better for curative and the chloride for prophylactic purposes. The concept of what may be called "depot prophylaxis" is potentially applicable to any drug if a salt or complex that is only slowly absorbed can be made, and this is really what Williamson & Desowitz (1956) have done in their work on combinations of Bayer 205 with Ethidium, Berenil, Antrycide etc. (see below).

Secondly, although the prophylactic properties of Antrycide are far from all that can be desired they have served to show that chemoprophylaxis of cattle trypanosomiasis in Africa can play a very important part in the development of that continent.

Antrycide as a curative drug. I.

Initial field experiments (Davey, 1950) were concerned with the treatment of T. congolense and T. vivax. It was shown that 0.5 mg./kg. subcutaneously [All treatments with Antrycide mentioned in this review were made subcutaneously unless otherwise stated.] of the methylsulphate cured all of 6 cattle infected with a Uganda

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* marketed by Imperial Chemical Industries Limited, Pharmaceuticals Division, England.

(Mubende) strain of T. congolense, 1 mg./kg. cured all of 7 cattle infected with a Southern Sudan strain and all of 9 infected by the bite of tsetse fly with a Kenya (Mariakani) strain. With a second Kenya strain (T.90 of the Kabete laboratory) 2 mg./kg. cured 10 of 11 animals. Experiments with T. vivax were more limited, but 1 mg./kg. cured 5 of 6, and 5 mg./kg. cured all of 5 cattle infected by the bite of tsetse fly with a virulent Kenya strain, and in Uganda, using a much less virulent strain, all of 6 cattle were probably cured with 5 mg./kg. On the basis of these results 5 mg./kg. was suggested as the dose to be tried in the field in cattle which might be infected with T. congolense and T. vivax.

Confirmation of its efficacy came from the Republic of the Sudan (Evans, 1950), Portuguese East Africa (Da Cruz Ferreira & Tendeiro, 1950), Italian Somaliland (Pellegrini & Bonelli, 1951), the Belgian Congo (Schoenaers, 1950; Andrianne, 1952) and particularly from Uganda where, since 1950, more than three-quarters of a million cattle have been treated, (annual reports of the Veterinary Department 1950, 1951, 1952 etc.). It was used, too, in Nigeria, Northern Rhodesia, Kenya etc. and no serious criticisms of its therapeutic effect were made (annual reports of the Veterinary Departments 1951 et seq., and of the West African Institute for Trypanosomiasis Research 1952 et seq.). Summing up, the recommended field dose is almost certain to cure T. congolense, and most cases of T. vivax.

T. congolense infections have also been cured in dogs and horses with 3–5 mg./kg. (Pellegrini & Bonelli, 1951; personal observations), and T. vivax in horses with 5 mg./kg. (personal communications).

Antrycide as a curative drug. II.

The laboratory experiments in small animals (Curd & Davey, 1950) had shown that Antrycide possessed a very marked action against such other species of trypanosomes as T. evansi, T. equinum etc. and the methylsulphate was therefore tried against them in the field. Camels infected with T. evansi have been cured with 5 mg./kg. in the Republic of the Sudan (personal communication from Mr. J.T.R. Evans), in Italian Somaliland (Pellegrini & Bonelli, 1951), and in Iran (Rafyi & Maghami, 1953). The same species has been shown to be susceptible to Antrycide in horses and dogs in Indo-China (Chary & Bocquet, 1955), but repeated treatments were used. In India, 5 mg./kg. cured T. evansi in horses, and 3 mg./kg. was completely satisfactory in cattle (Ray et al., 1953). Turton (1953) eradicated an outbreak of T. brucei in a group of horses at Accra with 5 mg./kg. repeated once more after 4 days. Zottner (1952), working in Morocco, regarded 5 mg./kg. as completely effective against dourine (T. equiperdum). T. simiae was cured in three pigs with 5, 4 and 3 mg./kg. respectively in Uganda by Wilson (1949b), but in Nigeria Unsworth (1952) failed to achieve complete cure of this infection with 5 mg./kg., relapses occurring three to four weeks after treatment.

Antrycide as a prophylactic drug. I.

The first field experiments with Antrycide (Davey, 1950) were also concerned to show that practical use could be made of the prophylactic properties of the chloride for protecting cattle in tsetse areas. They demonstrated that (a) a single dose prevented

animals from acquiring infection with trypanosomes for some months; (b) better protection was afforded against T. congolense than T. vivax; and (c) the very important point that trypanosomes "breaking through" the waning concentration of drug towards the end of the prophylactic period could emerge resistant to the drug, and therefore the important corollary that, in practice, re-treatment must be made soon enough to prevent the emergence of break-through strains.

The methylsulphate, because of its conversion in part to the chloride in the subcutaneous spaces, was also shown to have some prophylactic properties, and the salt has actually been used sometimes for prophylaxis. For example, in Northern Rhodesia (annual reports of the Veterinary Department 1952, 1953) some cattle have been kept in a fly belt for as long as 30 months free from trypanosomes by the injection of 5 mg./kg. methylsulphate every two months.

As a result of the first field trial it was recommended that for prophylactic purposes Antrycide should be used in the form of a mixture containing three parts by weight of the methylsulphate and four parts by weight of the chloride, a mixture called Antrycide prosalt. The chloride is there to give protection and the methylsulphate to eradicate trypanosomes that might be present at the time of treatment. The dose is measured in terms of the methylsulphate at 5 mg./kg.

The field work in Africa was continued by Fiennes (1953a, b) and Unsworth & Chandler (1952). Information is contained also in various annual reports written in Africa since 1950, particularly those of the Veterinary Departments of Uganda, Kenya and the Republic of the Sudan, and of the West African Institute for Trypanosomiasis Research. It would take too long to trace in detail the history of Antrycide through all the investigations, and I have therefore tried to summarize what seems important in the following conclusions. In framing them I have been helped considerably by conversations and discussions from time to time with workers in Africa.

(i) The period which may be allowed between treatments, if the cattle are to be kept completely free from trypanosomes, is dependent on the density of the tsetse fly population. If the "challenge" to the drug is anything but light the period between treatments must not exceed two months, and if the challenge by T. vivax is heavy some infections usually appear after a time even with this frequency of treatment. Unfortunately, there is no universal agreement upon what constitutes "light", "medium" and "heavy" challenges. The first quantitative work on the subject was done by the Kenya Veterinary Department, whose members have rendered a great service by always measuring fly density and natural incidence of trypanosomiasis in their experimental areas and correlating their prophylactic results with it. The density is expressed as the apparent density (AD), which is the number of tsetse flies caught per 10,000 yards of patrol, using a standardized catching technique. In the case of the tsetse fly Glossina pallidipes an AD less than 10 allows the area to be described as offering a light challenge, and one greater than 40 a heavy challenge. Under light challenge, prophylaxis with Antrycide prosalt at two-monthly intervals gives near-perfect results and it is possible that the interval

could be increased a little or the amount of chloride in the mixture reduced. Indeed, in an area with an AD less than 3, the prosalt at three-monthly intervals has given complete prophylaxis (personal communication from Kenya Veterinary Department). Under medium challenge, treatment every two months still gives excellent results, but it would be dangerous to increase the period, and at AD's approaching 40 some infections may appear after about a year's exposure. Under very heavy challenge T. vivax or, much more rarely, T. congolense may break through after a variable period of four to eight months, depending on the actual fly density.

Dr. E. F. Whiteside, who has emphasized for some years the important part played by the nature of the challenge in determining the efficacy of prophylaxis (it is apparent, too, in the different results obtained by Unsworth & Chandler in a "palpalis area" and a "morsitans area") thinks the drug is soaked up, as it were, if large numbers of trypanosomes are injected (personal communication) and I have some evidence that this may be part of the explanation. In experiments in mice and rats (unpublished observations) I have been able to show that the magnitude of the curative dose of certain drugs for T. rhodesiense is determined by the number of trypanosomes present at the time of treatment. In other words, the more trypanosomes there are present, the more drug is required to cure.

The magnitude of the challenge is clearly an important factor, and knowledge of it helps to explain why laboratory results in cattle are better than the results sometimes obtained in tsetse areas, and why there is outstanding success in some areas and relative failure in others. One might go so far as to say that all workers contemplating the occupation of tsetse country by cattle should first measure the density of flies present, even if roughly, because they will thereby be much more able to weigh the chances of success.

(ii) T. vivax breaks through sooner than T. congolense (T. brucei may also break through as early as T. vivax but probably rarely matters in cattle), and better protection can therefore be expected in a predominantly congolense area than in a predominantly vivax one.

(iii) The species of tsetse fly does not seem to influence the results, except in so far as different species are differently attracted to cattle (which affects the challenge) and to man (which affects the AD observed).

Antrycide as a prophylactic drug. II. Some uses in cattle

(i) In French West Africa, the Gold Coast, Nigeria and the coastal area of Kenya the cattle-raising areas of the north are separated from the markets of the south by tsetse belts. It was the habit in the past to force march the cattle past the danger with, of course, a resulting loss of condition made worse, sometimes, by infection with trypanosomes. The benefits to be derived from protecting the cattle with Antrycide have been demonstrated by Mornet et al. (1951, 1952), Unsworth & Birkett (1952) and workers in Kenya (annual report Veterinary Department, 1954).

(ii) In Uganda (Western Ankole) injections with Antrycide prosalt

every two months are used to protect cattle taken into a tsetse area to resettle it. The cattle (more than 7,500) have now been there for almost two years and are flourishing (personal communication from the Uganda Veterinary Department).

In Kenya an apparently successful experiment has been described of taking cattle from the normal grazing grounds during the wet season and putting them in tsetse areas, so allowing the grazing a chance to recover. The cattle were kept in the tsetse area under the protection of Antrycide (annual reports of Department of Veterinary Services, Kenya, 1953, 1954). The same reports speak of a ranch being maintained in a tsetse area with the help of Antrycide. Cattle for special purposes, e.g. milking herds and working oxen have also been kept in tsetse areas in the Republic of the Sudan (annual reports of the Veterinary Department, 1951–53).

Antrycide as a prophylactic drug. III.

Zottner (1952) considered that two doses of Antrycide prosalt should keep horses completely free from dourine (the breeding season is March to June) in Morocco. Ray et al. (1953) and Ramanujachari & Alwar (1955) suggested that the drug should be of service in protecting cattle and other animals against surra in India.

Other observations on Antrycide

"The blood of Antrycide-treated cattle drawn up by tsetses at frequent intervals does not destroy T. vivax which has established itself in flies; nor does it affect development of the trypanosome to the mature, infective stage; not even after 18–19 meals over a period of 41 days, which is believed to be longer than the life of a tsetse in nature." (Lewis, 1949). Similar conclusions were drawn by Roubaud (1952).

The toxicity of Antrycide

The chloride when injected subcutaneously or intramuscularly is virtually without general toxicity because it is so poorly soluble and seems to be absorbed only as a solution. It is of equal toxicity with the methylsulphate when given intravenously (Curd & Davey, 1950). It probably causes some local irritation when given subcutaneously, and the methylsulphate certainly does, but less than the phenanthridinium compounds. When the two salts are used together as the prosalt the chief local trouble is the "lump" of unabsorbed material which may persist when the small oedematous swelling caused by irritation has subsided. The risk of a lump is lessened if the injection site is massaged at the time of treatment, and sometimes it is dispersed by the movement of the animal. Most people prefer to make the injection in cattle behind the shoulder, but workers in Kenya have pointed out the advantages of making it into the dewlap where the risk of an unsightly blemish is minimal, and damage to meat or hide is negligible.

The maximum amount of the methylsulphate by itself or as it is contained in the prosalt which should be given to any species of animal subcutaneously or intramuscularly is 5 mg./kg. Even at this dosage a proportion of animals exhibit symptoms — increased salivation, sweating, uncontrolled

tremors etc. — suggesting stimulation of the parasympathetic system. The symptoms may be very marked in horses, which should be treated with great care. Climate seems to have an influence. I treated two horses in the relative cool of Kabete, and six (with Mr. T. W. Groves) in England and observed only slight side effects, but much more severe reactions have been described in Nigeria. A similar contrast occurs with cattle. Mr. W. T. Harrow and I dosed ten cows in England with 20–25 mg./kg. and only one died; the same dose in Africa might easily have killed them all. Under such conditions of strain as very poor health, over-heating, fright or undue exercise, 5–7.5 mg./kg. may kill cattle (Davey, 1950; Goodwir & Unsworth, 1952). Death under these circumstances does not usually occur until 10 days to 3 weeks later. The symptoms and lesions are characteristic and similar to those produced by doses of 10–15 mg./kg. which may kill without superimposed strain (Wilson, 1949a; Garner, 1950; Burdin & Plowright, 1952b; Andrianne, 1952). They include a haemorrhagic gastro-enteritis with or without zebra markings of the large intestine, pulmonary oedema, excess fluid in the thoracic and pericardial cavities, and evidence of serious damage to the kidneys.

The risk of accidents is, of course, more during the hurly-burly of mass treatments than during the treatment of a few individual cases and the general recommendation, following a lead given by Uganda, for the treatment of cattle in the field is now 4.5 mg./kg. (2 ml. of a 10% solution per 100 lb. body weight).

Intravenously, 5.9 mg./kg. killed one bovine within 5 minutes, and 5 mg./kg. given slowly caused severe shock in another (Shone, 1954).

COMPLEXES OF SURAMIN WITH THE PHENANTHRIDINIUM COMPOUNDS AND WITH ANTRYCIDE

It was pointed out above that the concept of "depot prophylaxis" is potentially possible with any trypanocidal drug if a poorly absorbed salt or complex can be made. Williamson & Desowitz (1956) have made such complexes of certain drugs with suramin. Suramin is exceptional amongst drugs in being acidic and it will combine with basic substances such as Pentamidine, Berenil, the phenanthridinium compounds, Antrycide etc. to form relatively insoluble complexes. Williamson & Desowitz have shown that certain of these complexes, particularly the complex of suramin with Ethidium and to a lesser extent with Antrycide, have marked prophylactic properties against fly-transmitted T. congolense and T. vivax when given to cattle subcutaneously. More than 7 months' protection was obtained with the Ethidium complexes in these preliminary experiments and clearly the results of field experiments will be awaited with great interest.

DRUG RESISTANCE

It is relatively easy, particularly in the larger animals, to make trypanosomes resistant to the drugs by undertreatment. Unfortunately, all the substances mentioned above, except Berenil, share some common factor which makes for cross-resistance amongst them. Sometimes the cross-resistance appears incomplete, but this may be more apparent than

real. For example, few workers would care to use more than 1.5 mg./kg. of Dimidium routinely and this is about the minimum curative dose for some strains of T. congolense. Now Antrycide will cure several, if not most strains of T. congolense at 1 mg./kg., and the advised field dose of 5 mg./kg. might therefore easily cure a strain of T. congolense partially resistant to Dimidium even if cross-resistance occurs, whereas the same cross-resistance would preclude the use of Dimidium (and possibly even of Ethidium at 1 mg./kg.) against a strain partially resistant to Antrycide. In any event, cross-resistance does exist, and care should therefore be taken in the use of all the drugs. Drug-resistance has not yet proved a problem of any magnitude, perhaps because care has been taken, and Unsworth (1954c) was able to say: "In spite of the extensive use of the drug in the field [i. e. Antrycide in Nigeria] ... there is as yet little reliable evidence that such has led to the creation of antrycide-fast strains of trypanosomes." The same statement could equally well be made of Antrycide in Uganda. Nevertheless, strains resistant to Dimidium have been picked up in the field because the recommended dose of this drug did not always cure (see, e.g. Wilson. 1950), which is indicative that the hazard ought not to be forgotten.

Drug-resistance can develop from inadequate prophylaxis as well as from inadequate treatment. As was remarked above, trypanosomes breaking through the waning concentration of Antrycide towards the end of its effective period may emerge with resistance to the drug. This will almost surely be true, too, of the newer prophylactic drugs if they come to be used, and so again, if prophylaxis is attempted, every effort should be made to make it complete. Examples of drug-resistance, and how it is developed, will be found in the work of Wilson (1949a, 1950, 1953), Davey (1950), Fiennes (1953a), and in the annual reports for 1953 and 1954 of the Veterinary Department, Kenya.

SOME RECOMMENDATIONS REGARDING THE USE OF THE DRUGS

The treatment of cattle trypanosomiasis

A possibly important point which bears on this subject must first be mentioned. It is so-called cryptic trypanosomiasis in which a cryptic focus of parasites is said to exist in cattle under certain conditions, and is supposed to be refractory, or more refractory, to treatment (Fiennes, 1950, 1952, 1953a, b). One gets the impression that something analogous to the leishmanial form of T. cruzi was sometimes suspected to be present, though no unequivocal evidence is given. Trypanosomes, mostly in a state of lysis, have been described in the capillaries of the heart and kidney (Fiennes, 1952); but is not the description "cryptic focus" — implying a precise and localized reservoir of parasites that are protected by their station, or by virtue of being different from the blood form — too suggestive without further evidence?

From a chemotherapeutic viewpoint it is important to know whether trypanosomes in sites other than the circulating blood are different or are sheltered from the action of most drugs as they would be, for example, if they were in the nervous system. We do not know all the details of the

pathology of trypanosomiasis in cattle that we should, but the weight of evidence from the field does not suggest that chemotherapy is fighting any mysteries. The curative doses of all the drugs now used were first worked out using infections only a few days old and they are being successfully used against much older ones. Actually, Randall & Laws (1947) produced evidence that Dimidium is possibly more effective in infections of long standing (up to 117 days) than in those existing for only a very short time before treatment is made. And again, if prophylaxis with Antrycide does not keep an animal entirely free from infection, but makes for a cryptic infection, how can one explain the results of, for example, Unsworth & Chandler (1952) who took such pains to demonstrate infection in cattle kept for almost a year in tsetse areas, and who failed; and how can one explain so many results now being achieved year by year in Africa?

Incomplete treatment can suppress an infection, and imperfect prophylaxis could lead to a similar effect, but are the resulting chronic infections (forgetting drug-resistance) qualitatively different from those we may call acute or semi-acute? If they are not (and there is little evidence to the contrary) then existing drugs should be satisfactory. In any event, one hopes that workers in the field will bear the problem in mind and record any unusual results they may obtain.

Regarding the modern treatments of T. congolense and T. vivax in cattle the choice appears to lie between Ethidium and Antrycide methylsulphate. If Berenil has advantages, more work is required to demonstrate them. It should be remembered, however, that Berenil may be of value for treatment of cases resistant to Ethidium and Antrycide. Individual cases in cattle may be treated according to the species present; for the purpose of mass treatment it should always be assumed that both T. congolense and T. vivax are present.

T. congolense and T. vivax in other hosts

Ethidium does not appear to have been tried in hosts other than cattle. Antrycide has been used to cure T. congolense in horses and dogs (3 mg./kg. should be ample) and T. vivax in horses (5 mg./kg.).

T. simiae in pigs

Unless Ethidium is better than Dimidium the drug of choice is Antrycide, and this may not be completely effective as a single-dose treatment.

T. evansi, T. equinum and T. equiperdum in horses

Antrycide is less toxic than suramin for horses and the treatment is simpler. The full dose of 5 mg./kg. need not be used, except possibly for T. equiperdum.

T. evansi in cattle

Antrycide (3 mg./kg.) has given very good results in India.

T. evansi in camels

Both suramin (better tolerated by camels than by most domestic animals) and Antrycide are effective; the choice seems to be an individual one.

Prophylaxis

Antrycide as the prosalt is the only drug which has received extended use for this purpose. It can be used to protect animals against T. congolense, T. vivax, T. evansi (and probably T. equinum) and T. equiperdum.

THE FUTURE

The search for new drugs continues. From a curative point of view all that can be done is to find something which will not bring about cross-resistance with existing drugs, and with which there will be good latitude between the curative dose and the dose producing undesirable side effects. The task is not an easy one because the modern drugs are good, very good when one considers what is asked of them, particularly that they should cure in a single dose.

But a new drug, even a wonder drug, will not now contribute much to the development of Africa unless it has, or can be given, prophylactic properties of a high order. The development of Africa demands that the rule of the tsetse fly be broken, that people and domestic animals shall occupy tsetse country, and in the battle chemoprophylaxis can play a big part. Antrycide is already helping much, but anyone called upon to inject thousands of cattle every two months knows its major defect only too well, and so the promise of longer duration of prophylaxis given by R. D. 2801, and the complexes of Williamson & Desowitz, is most encouraging.

Promise of perhaps even greater import has come from another quarter. Soltys (1955) describes an experiment in which 18 cattle were kept in a tsetse area for 28 months under the protection of Antrycide prosalt given every two months. The challenge was mainly from T. congolense transmitted chiefly by Glossina austeni. At the end of this time they were divided into three groups with six animals per group. Group I continued to receive the drug for a further 18 months and stayed in the tsetse area. Group II also stayed in the area, but drug treatment was discontinued. Group III was taken to Kabete, the drug treatment discontinued, and the animals kept there for 10 months under fly-proof conditions before being transferred back to the tsetse area where they were still not given drug treatment and where they stayed for 8 months. None of these animals developed trypanosomiasis (5 controls, introduced at the same time as Group III, contracted T. congolense within 2–3 weeks) and none could be infected even artificially.

The immunity developed by the animals, particularly striking in those of Groups II and III, is clearly a solid immunity, and not "the kind usually called premunition; where the condition of resistance is accompanied by or even dependent on the survival of the parasite," (Hornby, 1949).

Fiennes (1953b) used Antrycide to aid the development of apparently effective premunition in calves born and reared in tsetse country, but as Hornby (1949) pointed out, research on premunition finds qualified support because the animal remains a reservoir of infection. The results obtained by Soltys, if confirmed, are clearly of much greater practical significance and it is most important that his experiments should be repeated. This is being done in East Africa (annual report of the East African Tsetse and Trypanosomiasis Research Organization, 1954–55) and in the Republic of the Sudan (personal communication).

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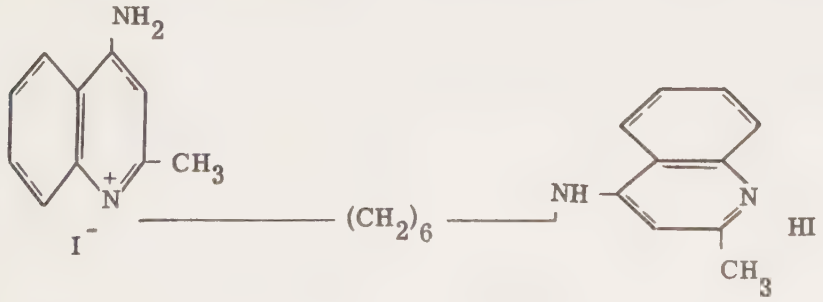
ADDENDUM

Tozocide

Since this review was written and while it was in the hands of the editor, a new substance called Tozocide* has been described (Austin et al., 1957). It has the constitution:



and is the hydriodide of 6'(4-quinaldylamino) hexyl-4-amino quinaldinium iodide. Like Antrycide, it owes something to the work of Iensch (1937),

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* Allen and Hanburys Ltd., Ware, England.

but it is unusual, as a trypanocidal compound, because of the position of the linkage between the two ring systems.

Laboratory work with infections in mice has shown that Tozocide is more active than Antrycide against T. congolense and T. vivax, and that it has some prophylactic properties (Austin et al., 1957). Preliminary tests in Kenya have indicated that activity is also obtained in cattle, but details are not available (personal communication from Dr. H. O. J. Collier).

Saline Content of Drinking Water for Livestock

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INTRODUCTION

The problem of the maximum safe concentrations of dissolved salts in the drinking water of livestock is one which has received very little attention. This is surprising in view of the comparatively high saline content of waters upon which millions of livestock subsist for many months of the year in various countries which are situated in arid or semi-arid regions. Such regions constitute large areas of North and South Africa, North and South America, the Middle East, Asia and Australia. Underground waters drawn from wells or from sources tapped by bores of various depths are frequently very saline, the principal salts being the chlorides, sulphates, carbonates or bicarbonates of sodium, calcium and magnesium, with sodium chloride usually predominating. In extreme cases the waters are from deep bores associated with oil wells and may then contain very high concentrations, up to 20%, of dissolved salts. Saline water may also occur in rivers which have become polluted with effluents from mines or factories or by salt water escaping from oil wells. Traces of fluoride also occur in many waters, but these will not be considered here as a number of excellent reviews on the subject have been published during the past 20 years.

It is proposed to discuss here the information, published and unpublished, which has come to the notice of the reviewer and which has been derived from the three following sources, (a) experiments, (b) clinical observations following losses of stock traceable to highly saline waters, and (c) answers to questionnaires sent by investigators to extension workers and stock owners.

The paucity of the available information was the main reason why two years ago this Division of the Commonwealth Scientific and Industrial Research Organization in Australia commenced a series of experiments to determine the tolerance of sheep to saline waters.

ANIMAL EXPERIMENTS

Sheep

The earliest experiments appear to be those conducted by a committee appointed by the Australian National Research Council (Seddon, 1926, 1927). The experiments, which involved only a few animals and were conducted for periods varying from 4 weeks to over one year, indicated that sheep could tolerate water containing up to 2.4% sodium chloride,

1.3% magnesium chloride or 0.5% magnesium sulphate. However, in view of the limited number of animals used, the Committee did not prepare a statement for publication.

Heller (1933) experimented with groups of 5 sheep for periods of only 6 weeks. The results showed that under the conditions of the experiment the animals could exist on waters containing 2.5% NaCl, 2% $MgSO_4$ or 2.5% $CaCl_2$, but the author suggested that 1.5% of total solids should be considered the upper limit under which maintenance could be expected. He also showed that a period of a week or more usually elapsed before sheep whose drinking water was changed to a saline one would resume normal consumption of water.

Velu (1936) offered to groups of sheep fresh (city) water continuously, a naturally occurring Moroccan water containing 1.2% total salts, including 0.64% chlorides as NaCl and 0.17% magnesium as MgO, continuously, or the fresh and saline waters alternately. No adverse effects were observed during the year of the experiment among the sheep which drank the saline water and their weight increase was the same as that of the control animals which were offered the city water to drink.

In the first experiment conducted by the author (Peirce, 1957a) the effects of various concentrations of sodium chloride in the drinking water of groups of 6 sheep were studied for a period of 15 months. It was found that the animals receiving 1% NaCl did as well as the control sheep (receiving rain water); that a concentration of 1.5% NaCl was slightly toxic; and that a concentration of 2% was definitely so. Several of the animals receiving the highest concentration, however, showed some degree of adaptation to the treatment after a few months. In the second experiment (Peirce, 1957b) a concentration of 1.3% NaCl was assumed, from the results of the first experiment, to be safe, and such water was compared with similar water in which various concentrations of $MgCl_2$ from 0.02 to 0.5% replaced equivalent concentrations of NaCl, rainwater again serving as the negative control. Many of the sheep refused at first to drink these waters and hence the concentration of salts was gradually increased, over a period of about 2 months, from zero to the desired levels. Even then about 2 animals on each treatment did not consume food and water satisfactorily and had to be replaced by others. Thereafter for the remaining 10 months of the experiment, concentrations of up to 0.1% $MgCl_2$ (together with 1.2% NaCl) appeared to be quite innocuous. Those of 0.2% and 0.5% MgCl were found to be somewhat toxic in that one of the 6 sheep in each group receiving such waters became very ill.

Cattle

Seddon (1931), working with one calf (110–150 kg. live weight) on each of four concentrations of NaCl, found that the animals were able to tolerate up to 1% for a period of 6 months without showing any ill effects. However, the weight increase of the calves receiving 0.4% or 1% NaCl was less than half that of the control calf or the calf receiving 0.14% NaCl.

Heller (1933), using a few cows for periods of up to 5 months, found that some animals which were not in production could tolerate 2% NaCl. However, 1.5% seemed to be the maximum concentration which could be considered safe for maintenance. The maximum safe limit for lactating

cows was apparently somewhat lower. Calcium chloride was found to be more toxic than sodium chloride and the lowest level tested, 1%, was not tolerated by the animals. A saturated solution of calcium sulphate was readily consumed once the animal became accustomed to it and no ill effects were observed.

Pigs

Seddon (1931) carried out brief experiments with a few pigs and found that one mature animal tolerated 2.5% NaCl for 40 days, although it showed some signs of nervous derangement at the end of the experiment. Two other pigs (120 lb. live weight) consumed water containing 1.9% NaCl for 13 and 22 days, respectively, and one young pig (28 lb. live weight) consumed water containing 0.9% NaCl for 40 days without showing any ill effects; the last pig doubled its weight during the experiment.

Heller (1933), in experiments with groups of 2 or 3 pigs for periods of 5 or 6 weeks, found that 1.5% NaCl was toxic for pigs, particularly young ones, but that both young and old pigs could tolerate 1% NaCl and grow as well on it as on fresh water.

Bohstedt & Grummer (1954) conducted an experiment which was a deliberate attempt to produce salt poisoning in pigs rather than to determine the maximum concentrations in the drinking water which would be tolerated. The animals received swill containing either 1.5% or 2% salt but no additional drinking water. Seven of the 10 animals died or were killed in a semi-comatose condition within 5 days.

CLINICAL OBSERVATIONS

Sheep

Legg (1929) reported a mortality of about 30% in a flock of 200 travelling ewes and rams, presumably from salt poisoning. The animals were removed from railway trucks after having travelled about 400 miles. They were thirsty and apparently drank from feeding troughs which had previously contained salt, but, owing to heavy rain having fallen shortly before, apparently now contained a concentrated solution of sodium chloride.

Moule (1945) also reported a mortality of 2%, with many more sick animals, in a flock of 3,000 travelling wethers which had been allowed to drink, following a day without water, from a trough containing water with a salt content of 2.4%.

Cattle

Scott (1924) investigated the death of some cattle and the illness of others and concluded that the troubles resulted from drinking from a waterway which was polluted by the effluent from a salt works, giving a concentration of 1.7% NaCl in the water. He confirmed the tentative finding by offering this water to two cows. They lost an average of 180 lb. in weight in 14 days, compared with a gain of 88 lb. by two similar beasts given tap water to drink.

Ohman (1939) reported the death of about 7% of a herd of 200 cattle within 9 days of a change of water from a surface supply (presumably salt

free) to bore water containing almost 1.5% NaCl.

McConnell (1945), in a general article, drew attention to the fact that over a period of years many cattle and other livestock have become poisoned, at least in Oklahoma, owing to pollution of their water supply by saline discharges from oil wells. He listed the symptoms of salt poisoning and suggested some methods of treatment.

Pigs

Buffagni (1935) reported an outbreak of poisoning in which a number of pigs became ill and five died after having received large quantities of brine mixed with their food. It was estimated that each pig ingested 500–600 ml. of a 20% solution of NaCl per day, on three successive days.

INFORMATION FROM QUESTIONNAIRES

In the absence of precise information from experiments, a number of authorities have endeavoured to obtain information from livestock owners, veterinary workers and other interested persons by means of questionnaires. This method of approach has been used especially in Australia, but also to some extent in Africa. It suffers from the disadvantage, however, that many of the opinions expressed are biased by the particular person's own views. Hence care is necessary in drawing up any recommendations regarding toxic concentrations from such information.

Australian enquiries have led to the promulgation of separate recommendations for each State for maximum concentrations of salt which can be tolerated, and these differ widely from State to State, and sometimes even among authorities within one State. These recommendations are:

New South Wales (Ramsey, 1924):
Sheep, 1.7% NaCl, 1.9% total salts.
Cattle, 1.1% NaCl, 1.4% total salts.
Horses, 0.9% NaCl, 1.3% total salts.

Queensland (Brünnich, 1927):
Sheep, less than 0.9% NaCl.

South Australia (Jack, 1930):
Sheep, 1.6% total salts.
Cattle, 0.9% total salts.
Horses, 0.6% total salts.
} containing about 80% NaCl.

South Australia (Spafford, 1941):
Sheep and Beef Cattle, 1.0% total salts.
Dairy Cattle and Horses, 0.7% total salts.

Victoria (Slade, 1921):
Stock, 0.9% salt.

Western Australia (Mann, 1921):
Livestock, 0.9% salt.

Western Australia (Simpson, 1928):
Sheep, 1.3% total salts, 0.05% Mg.
Cattle, 1.0% total salts, 0.04% Mg.
Horses, 0.65% total salts, 0.03% Mg.

In all cases it has been pointed out that the concentration which can be tolerated will depend not only on that of the most abundant constituent (sodium chloride), but also on that of the other constituents as well; with the suggestion that divalent cations (calcium and magnesium) and anions (sulphate and carbonate) may be more toxic than monovalent cations and anions (sodium and chloride).

Velu (1936) also issued questionnaires to veterinarians in Morocco and from the answers concluded that all animals safely consumed brackish waters up to a certain concentration. In the article he did not state definitely this maximum concentration, but did give one instance of a satisfactory water which contained 0.9% NaCl. Furthermore, he observed oxen and mules which drank for more than one year water containing 0.9% total salts and 0.7% NaCl. He states that these animals were vigorous and in excellent health.

On the other hand, Steyn & Reinach (1939) in South Africa, suggested much lower levels as maximum safe concentrations, basing their statements on information supplied by farmers and stock inspectors. They concluded that water containing as little as 0.5% salts was toxic for stock. Most of the evidence for this belief, however, appeared to depend on the development of diarrhoea alone, a condition which could be caused by one or more of a number of other factors, such as internal parasites, pica, poisonous plants and so on. It is noteworthy also that many of these waters contained rather high concentrations of fluorides, 4 p.p.m. or more.

THE CLINICAL AND BIOCHEMICAL EFFECTS OF WATER OF HIGH SALINE CONTENT

Probably the most noticeable effect of water of high saline content on cattle, sheep and pigs is a decreased food consumption (Scott, 1924; Heller, 1933; McConnell, 1945; Peirce, 1957a). In many cases, indeed, salt injury is actually a condition of partial inanition, and the reduced increase in weight or the emaciated state and the diminished milk flow of animals observed by Scott (1924), Seddon (1931), Heller (1933), McConnell (1945) and Peirce (1957a) may have been due to a lessened intake of food rather than to a specific metabolic effect of the salt. It has not been established, however, whether the reduced food consumption is due to the effect of the salt on the flora of the alimentary tract or to some other cause.

Another sign which has frequently been observed is diarrhoea (Scott, 1924; Legg, 1929; Buffagni, 1935; Steyn & Reinach, 1939; McConnell, 1945). In one instance (Ohman, 1939) only a few animals were affected and in another (Peirce, 1957a), the condition was intermittent. Post mortem examination has usually revealed that this condition is accompanied by inflammation of the alimentary tract (Scott, 1924; Legg, 1929; Buffagni, 1935; Ohman, 1939; Steyn & Reinach, 1939; McConnell, 1945; Moule, 1945), the inflammation being extremely severe in many instances.

Another group of signs which have been frequently reported are those indicative of degenerative changes in the central nervous system. These have included stiffness of the legs or general incoordination of gait, paralysis of the hind quarters or even a general paralysis, muscular spasms, champing of the jaws, convulsions, blindness, weak pulse, laboured breathing and coma (Seddon, 1931; Heller, 1933; Buffagni, 1935; McConnell, 1945; Moule, 1945; Bohstedt & Grummer, 1954).

Other signs which have been reported by a single observer are profuse salivation, vomiting, elevated temperature, cystitis and nephritis (McConnell, 1945), anaemia (Buffagni, 1935), and abortion (Scott, 1924). In addition Steyn & Reinach (1939) suggested that the ingestion of saline waters leads to the formation of urinary calculi in animals. The causes of urinary calculi are, however, many and varied, and it does not appear to have been the experience of other workers that the formation of urinary calculi is one of the main symptoms resulting from the drinking of saline waters. The author (Peirce, 1957a) in fact did not find any calculi in four sheep which had drunk continuously for 15 months water which consisted of either 1.5 or 2% NaCl. On the other hand indeed, it has been the experience of this laboratory that urinary calculi frequently develop in wethers fed in pens and receiving good quality water, that is, water of low saline content.

Intense thirst of animals drinking water of high saline content was reported by McConnell (1945) and increases in the intake of water with increasing concentration of sodium chloride in the drinking water, at least by sheep, were observed by Seddon (1926), Heller (1933) and Peirce (1957a). Increases were not observed in cattle (Seddon, 1931) nor in pigs (Heller, 1933), and in the case of sheep a concentration was eventually reached at which the intake became reduced or actually ceased (Seddon, 1926; Heller, 1933).

Along with an increasing intake of water there is an increased excretion of urine (McConnell, 1945; Peirce, unpublished data), and most of the sodium chloride is excreted by this route (Seddon, 1926; Peirce, unpublished data), a finding similar to that of Heller & Haddad (1936) with rats. Some of the salt, however, may be stored in the muscular tissue (Seddon, 1926).

The effect of water of high saline content on the electrolyte concentration of the blood of animals has been found to be relatively slight. Heller & Paul (1934) did indeed observe increases of 10 to 20% in the Na and Cl concentrations in the plasma of sheep and cattle given 2% NaCl to drink and in that of pigs given 1.5 NaCl; in most cases the animals were very sick and died the day after these values were obtained. Peirce (1957a) observed increases in the chloride content only of the plasma of sheep which received 2% NaCl. These increases, of the order of 5%, were recorded throughout the duration of the experiment from the 5th week onwards. On the other hand, Seddon (1926) with sheep which received 1.7% NaCl, and Bohstedt & Grummer (1954) with pigs which were seriously affected by 1.5 or 2% NaCl, found no increases in the sodium or chlorine of the plasma or serum.

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Rinderpest: Some Notes on Control by Modified Virus Vaccines. II

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THE MODIFIED VIRUS VARIANTS OF RINDERPEST

II. Lapinized Virus

History

Early observations on the passage and attenuation of rinderpest virus in the rabbit were recorded by Edwards (1925, 1927); he injected ox blood and successfully maintained direct passage for more than a year. Had all his experimental rabbits not been destroyed by an epizootic, lapinized virus might well have preceded the goat-adapted virus in the history of these vaccines. (According to Edwards, the goat population of India and Ceylon suffered indirectly as a result of the development of goat virus; he was, of course, referring to the spread of rinderpest in goats following the injection of these animals in the field with partly attenuated rinderpest virus in order to obtain a supply of virus for vaccination of cattle. However, it is interesting to reflect upon the accident or providence, in all probability determined by the small numbers of goats available in Japan and Korea, which resulted in goat-adapted virus being evolved in India, where it was most needed, and lapinized virus in the Far East, where the cattle were so much more susceptible.) Other workers in this field met with some success (Jacotot, 1932; Inoue, 1934) while Cèbe & Perrin (1935) very successfully maintained two strains of rinderpest virus in rabbits for over 30 and 40 serial passages and noted that there was a reduction in virulence for calves. No serious attempt seems to have been made to exploit these researches until in 1938 Nakamura, Wagatsuma & Fukusho published the first of a series of papers on the experimental infection of rabbits with rinderpest, which have been continued by these Japanese workers until the present time (Nakamura et al., 1940–55). Working with the Canadian–American Research Group at Grosse Isle, Baker (1946) was able to adapt the Kabete "O" rinderpest virus by alternate passage in calves and rabbits but was unsuccessful with initial direct passage. Likewise, Haddow & Idnani (1947) in India, were at first unsuccessful until they used a fully virulent field strain of rinderpest from an epizootic; they maintained that full virulence was an essential requisite. This was to some extent confirmed by Walker (1947), who was unable to adapt Baker's lapinized virus of 65 serial passages to the egg but was able to do so with an early rabbit serial, "out-passaged" once in a calf; Walker attributed the first failures to an insufficient titre of virus for the threshold of infection necessary for the embryo.

Recognition of the work done by the Japanese was delayed by the second world war and because the reports on trials carried out in Korea were published in Japanese journals. The earliest records were concerned with Nakamura III virus and simultaneous injection of immune serum (Nakamura, Fukusho & Kuroda, 1943). Before this, however, the virus had been distributed to Mongolia where it was used in 1941 and during the war, to control rinderpest; the first published report of the successful use of lapinized virus was by Isogai (1944), who showed that it was safe and effective for Mongolian calves. Trials were also carried out in North China but records of field vaccination were not available until Cheng & Fischman (1949) presented the results of their researches to the International Rinderpest Conference, Nairobi, Kenya, in 1948. Immediate interest and recognition followed and the practical assistance of the Food and Agriculture Organization of the United Nations played a major role, both by testing the vaccine in the Far East and by initiating distribution of the Nakamura III strain of lapinized virus from the Nanking National Research Bureau of Animal Industry. Confirmation of the success of the vaccine was soon reported by Alton (1950) and by Watson (1950) in Hong Kong, in Egypt (Anon., 1950), by Brotherston (1951) in East Africa, by Datta & Dhanda (1951) in India, and by Fulton (1951) in the Gambia. The vaccine has since become very popular and, in the sections which follow, reference will be made to work in other parts of the world. It is possible, however, that indiscriminate use of this relatively mild vaccine, particularly in resistant cattle, may result in the method falling into disrepute. In Russia, lapinized virus as well as other forms of live rinderpest vaccines have been reported to be unsuitable for the conditions prevailing there (Bazulev, 1953). Recently, Iyer & Srinivasan (1954) adapted a Madras strain of rinderpest virus to the rabbit and have used it in buffaloes, but the virus in use in all other parts of the world has been derived from the Nakamura III strain of virus which had had at least 795 serial passages before it was distributed.

Susceptibility and resistance to the lapinized virus

All breeds of rabbits appear to be susceptible to the fully "lapinized" virus, but variations have been recorded. Animals under four months of age were considered by Brotherston (1951a) to give poor temperature reactions while Simpson (1954) noted that those below four and a half and over six months frequently failed to react. On the other hand, Scott & MacLeod (1955c) found that there was no significant difference between four, six and twelve-month-old rabbits but that those under four weeks were less susceptible as measured by temperature reaction, lesions and yield of virus. Sex has not been shown to have any influence although pregnant animals may be unsuitable. Mornet et al. (1953) recorded that 1% of rabbits were refractory in one way or another at Dakar and that atypical reactions were rare. Brotherston & Brown (1955) compared the lesions and yield of virus from highly inbred and randomly bred rabbits and found that the inbred animals appeared to be resistant and required at least 100 times the virus dose which would initiate the same yield from

randomly bred animals. Attention was drawn by Biggars & Claringbold (1954) to the routine use of inbred animals, which they suggested might well give less efficient results in biological assay apart from the well known disadvantages of decline in vigour and health. Further work is required on this aspect of susceptibility to virus diseases.

Cattle of different breeds were soon reported to vary in their reaction to the Nakamura virus. In Japan the black cattle were highly susceptible and, even after 860 rabbit passages, the Chinese and Korean substrains caused a high mortality. Korean cattle were very susceptible (Nakamura & Kuroda, 1942) whereas Mongolian cattle could be immunized safely by a virus of fewer rabbit passages (Isogai, 1944). European cattle reacted mildly although there were varying degrees of reaction according to the breed (Kwong & Lee, 1947). Zebu cattle in India and Africa generally showed little or no reaction. The indications are that the severity of the reaction to lapinized virus corresponds directly to the susceptibility of the breed to the natural disease.

Several workers have drawn attention to the enhanced susceptibility of young animals (Kwong & Lee, 1947; Alton, 1950; Watson, 1951). However, as such young animals offer resistance to the more virulent goat-adapted virus, the matter and its possible relation to acquired and colostral immunity requires investigation.

Buffaloes in India were susceptible (Haddow & Idnani, 1947) but were reported to react mildly or to offer more resistance than cattle in some parts of China (Cheng & Fischman, 1949). A lethal effect of virus on young Asiatic buffaloes was reported by Stevenin, Huard & Goeffon (1955), but it is not clear whether this was due to high susceptibility or to the effects of intercurrent trypanosomiasis.

Goats and sheep were susceptible and reacted mildly in India (Datta et al., 1951); several attempts have been made to produce a "reactor" vaccine from goats but, although a few passages have been accomplished, the virus has died out on continuation of the series.

Hudson, working with the indigenous pigs of Siam, found that they varied in susceptibility, but alternate and direct series were maintained (Hudson, 1950; Hudson & Wongsongsarn, 1950). Nakamura et al. (1954) noted that in pigs a viraemia and antibodies could be demonstrated but there was no visible reaction, while Bugyaki (1955) reported that in the Belgian Congo pigs were resistant.

Rinderpest virus which had been passaged alternately five times between rabbits and calves was adapted to the chick embryo by Walker (1947); his claim that this was a lapinized virus appeared to be justified, for after 29 passages in the egg, the virus was still able to protect against the parent rabbit virus and to immunize calves against a bovine strain of rinderpest. Nakamura, Sato & Miyamoto (1947) made many attempts to adapt the Nakamura III strain to the chick embryo; by intravenous inoculation they obtained multiplication in the embryo spleen but were unable to maintain it. Later, using virus of the 763rd rabbit passage, they carried out 11 alternate passages between the chorio-allantoic membrane and rabbits, followed by chick embryo passage by intravenous injection of embryo spleen. After 246 such inoculations, Japanese black cattle reacted mildly and were immunized (Nakamura & Miyamoto, 1953).

Lapinized virus has been cultivated in tissue culture using lymph node, spleen and bone-marrow of rabbits, and serial passage in lymph node tissue culture has been recorded by Takematsu & Morimoto (1954).

Virulence of the lapinized virus

Serial passage in the rabbit was associated with attenuation in virulence for cattle by those engaged in the early work. Nakamura et al. (1938), Nakamura (1940) and Cheng & Fischman (1949) first drew attention to the increase in virulence for the rabbit; the change was apparently very sudden for at serial passage 1–5 only 18% of rabbits showed lesions while between 6–10 only 7% were without them. At the 600th passage the virulence for the rabbit was considered to have become "fixed". Datta et al. (1951) and Simpson (1954) made a further 170 passages beyond the 795th and reported no alteration in reactions in either rabbits or cattle.

Nakamura et al. (1943) were unable to restore the virulence of the virus for cattle after 10 successive out-passages in cattle but Brotherston (1951a) by alternate passage demonstrated a decrease for rabbits and an irregular increase of virulence for cattle as indicated by thermal reaction and lesions. Ramon (1956) has criticized the use of live attenuated viruses in the control of rinderpest and has cited Brown et al. (1954), who indicated the risks of vaccinating susceptible animals which might react with unexpected severity. However, Brown and his colleagues were concerned with the reaction but were unable to recover virus from the cattle, and therefore the question of a back mutation or an increase in virulence of the immunizing agent did not arise in the incident they recorded. Iyer & Srinivasan (1954) returned a newly adapted lapinized virus of 178 passages to buffaloes for 16 consecutive serial passages and found that there was no alteration in virulence for buffaloes.

With passage in other species of animals, however, the results have been quite different. When Hudson & Wongsongsarn (1950) carried out their experiments in pigs, the virus caused a milder reaction in buffaloes than the parent virus and the immunizing titre was reduced. Nakamura & Miyamoto (1953) showed that the virulence for highly susceptible cattle was reduced, and that for the chick embryo distinctly increased; furthermore the pathogenicity for the rabbit was reduced. Although in these instances the serial passages were prolonged further than they have ever been in cattle, there seems to be good ground for suspecting that "re-adaptations" have been achieved, resulting in substrains of virus which might become fixed for the new hosts at a greatly reduced level of virulence for cattle. Confirmation for this in lapinized-avianized virus has been reported by Reisinger et al. (1954), who returned it to the rabbit for 220 passages without altering its effect upon Korean cattle which were highly sensitive to the parent virus. On the contrary Nakamura & Kishi (1954) found that, after 15 passages in rabbits, the "L-A" strain virtually reverted to lapinized virus and produced complement-fixing antigen only in rabbits. The picture is therefore confused and some interesting research can be envisaged by examination of the various strains and "substrains" of the virus.

Route of infection and transmission of lapinized virus

Rabbits are usually infected intravenously, but other routes can be used. Mumatzey & Koyamu (1945) even described successful infection in some cases by mouth and by the rectum. Contact infection in rabbits does not occur (Cheng & Fischman, 1949).

Cattle and other animals are usually infected subcutaneously, but Scott (1952) used the intradermal route and found that the immunizing dose could be reduced; the method was not recommended because of technical difficulties in the field. Cattle in contact with vaccinated animals for up to 21 days after injection of virus did not become infected and remained susceptible (Brotherston, 1951a).

Temperature reaction to the lapinized virus

Edwards (1925) noted a slight rise of temperature on the second and third day after intensive rabbit to rabbit passage. Nakamura et al. (1938) classified thermal reactions into four types in rabbits; a typical fever was a rise of 1.5° to 2° C. for two or three days, a slight fever was a rise of 1° C. for one or two days, and there were some animals which had irregular temperatures or no rise at all. Brotherston (1951a) described the normal temperature of rabbits as 101.6° to 102.4° F., and that over 292 observations the average temperature of infected rabbits was invariably over 104° F.; fresh material or blood always produced a quicker response than did freeze-dried tissue. Scott & MacLeod (1955) found that infection with less than 1,000 I. D. $_{50}$ doses of virus delayed the thermal reaction. Mornet et al. (1953) investigated this aspect of temperature reaction in some detail.

Cattle vary in their thermal reaction to lapinized virus; the breeds highly susceptible to rinderpest give temperatures comparable to those induced by goat-adapted virus; this may occur from the fifth to the tenth day after inoculation. Mild reactions have been described most frequently and these may be observed in a proportion of the animals only (Wells, 1951; Bugyaki, 1955): Simpson (1954) described a variation according to condition. European-zebu crosses were reported by Brotherston (1951b) to show little or no temperature; but Scott (1954a), by examining recordings of over 5,000 morning rectal temperatures from this type of animal, found a significant rise between the fifth and the seventh day after vaccination, but this was not readily detected in individual animals. The absence of visible reaction, so useful during anti-rinderpest control with goat-adapted virus, has been a disadvantage to those using lapinized virus in the field. Severe temperature reactions in a herd of Jersey heifers have been described by Brown et al. (1955).

Temperature reactions have been reported in buffaloes, goats, sheep and pigs.

Clinical signs other than temperature

General malaise and respiratory distress usually associated with

fever have been described in the rabbit, and diarrhoea was observed in some individuals by Baker (1946), although Mornet et al. (1953) considered that these unusual symptoms were rare.

In cattle, anorexia, lachrymation, cough and nasal discharge have been observed, with diarrhoea usually confined to calves, generally in very susceptible breeds. Some workers have described a reduction in milk yield (Kwong & Lee, 1947) but Brotherston (1951b) observed milk records of 100 cows for 25 days after vaccination, and showed that the average yield was not reduced. Without doubt, this very delicate index of health is a matter of individuality as well as breed and will vary according to the susceptibility of the animals. In many breeds and crosses of cattle, clinical symptoms have been entirely absent and this has been a disadvantage to the field workers who had no indication whether the vaccine was potent or even viable. On the other hand this feature was convenient in areas where farmers disliked any upset following vaccination, or where the risk of intercurrent disease was high, and perhaps has been responsible for vaccination of naturally resistant cattle as a matter of expediency when goat-adapted virus would have been more suitable.

Reactions in buffaloes, sheep and goats have been reported to be mild, with the exceptions already noted. Pigs in Siam occasionally showed anorexia and diarrhoea.

Pathology and histopathology

During the early researches with the rabbit, pathological changes were not observed. Fukusho & Nakamura (1940) were the first to describe lesions, which they observed after the first three serial passages, becoming more marked as the virulence for the rabbit increased. The lesions were inflammation and enlargement of the lymphoid tissues of the intestine (the Peyer's patches, the sacculus rotundus, the tonsilla caecalis, the appendix); the mesenteric lymph nodes were grossly affected and the spleen was enlarged. Later necrosis was marked and gave the characteristic appearance now recognized as pathognomonic, in which the lymphoid tissue became oedematous, greyish-white and granular. Histologically, the changes began at the 24th hour in the Peyer's patches and in all affected tissues and consisted of degeneration of the lymphoid cells and proliferation of the reticulo-endothelial cells of the germinal centres; necrosis began at the 48th hour and progressed for 3–4 days. Resolution started about the sixth day and regeneration was complete in 4–5 weeks. Baker (1946) noted inflammation of the mucous membrane of the stomach and intestines with formation of a membrane while Walker (1947) included enlargement of the Peyer's patches as a sign of infection. Cheng & Fischman (1949) and Brotherston (1951a) confirmed these findings with Nakamura III virus, and Brotherston described lesions in lymph nodes remote from the abdominal region. An excellent description with illustrations and photographs of the pathology has been published by Mornet et al. (1953). The fact that Nakamura III virus can be demonstrated positively in the rabbit by a disease syndrome is of great importance for future research.

The histopathology in cattle has recently been described by Thiéry (1956).

Mortality

Reports of the mortality in rabbits are few, presumably because they are killed at an early stage of infection for vaccine production; in the early passages the death rate was low but could be 80% in the presence of intercurrent disease, e.g. coccidiosis. In East Africa only two rabbits out of a total of 50 injected with virus survived for two months, the remainder having died from secondary infections following the severe necrosis.

In hypersusceptible cattle deaths can be very high, Nakamura & Kuroda (1942) reported 29% in Korean cattle and, with a highly lapinized virus of over 800 passages, mortality in Japanese black cattle was 43% (Fukusho et al., 1953). Reports of mortality in more resistant breeds of cattle have been rare although some losses have been reported after vaccination of calves (Kwong & Lee, 1947; Alton, 1950; Watson, 1950, 1951).

Some fatalities were reported in Asiatic buffalo calves by Stevenin et al. (1955).

Distribution of virus in the tissues

Assays carried out by Nakamura (1941) indicated that the highest concentrations were in the mesenteric lymph nodes. Scott (1954b) investigated the titre in dried tissues and found that the yield from the appendix was five times greater than from other tissues, but that the sacculus rotundus, Peyer's patches, spleen, mesenteric lymph nodes and tonsilla caecalis also had high concentrations. Virus was demonstrated in other organs, but brain, bile and urine contained none; the highest concentrations were over ten million infective doses per gramme of dried tissue, as tested by intravenous injection of rabbits. Mornet et al. (1955) described the titration of virus in fresh and freeze-dried tissues. A viraemia can be demonstrated in the rabbit, virus appears in the blood not earlier than 10–15 hours after infection and persists for 10–22 days; according to Nakamura the virus in the blood is attached to the leucocytes.

Titrations of virus in material to be used for vaccine are of great importance in ensuring that a reasonable standard is maintained. In the early experiences of Brotherston, sufficient attention was not paid to the errors inherent in titrations carried out on the basis of a 100% end-point using small numbers of rabbits or cattle. Under these circumstances, up to tenfold differences observed by workers using individual techniques might be more apparent than real and should not be taken too seriously. It is important, however, in laboratories where vaccine is produced, that a recognized standard be found for a basis of comparison of titrations carried out in rabbits and cattle. Employing these methods, Scott & MacLeod (1955a) found that an infecting dose of 1,000 $I.D._{50}$ produced the same reaction and yield of virus as 50,000 $I.D._{50}$ while Brotherston & Brown (1955) were able to show that inbred rabbits were more resistant than randomly bred animals and required 100 times the infecting dose to produce an equivalent yield.

Assays of vaccines prepared from rabbits have been carried out in cattle, and Mornet et al. (1955) reported that 150 rabbit infective doses

were the equivalent of one dose required to immunize an ox; this agrees quite well with the experiences in East Africa where about 100 rabbit doses were calculated to be the equivalent, on a 50% end-point basis.

A viraemia in cattle was demonstrated on the sixth and seventh days after vaccination by Brotherston (1951a) but the concentrations of the virus in the tissues of reacting cattle have not yet been investigated.

Viraemia also occurs in goats and sheep, but the "reactor vaccine" suggested by Cheng & Fischman has not been developed except in Siamese pigs. Hudson & Wongsongsarn (1950) found that in buffaloes the immunizing dose of pig-passaged virus rarely exceeded 5 ml. of a 1 : 20 suspension of spleen in blood diluted 1 : 1,000.

Resistance and viability of the virus

The resistance of lapinized virus in the tissues has received attention because these materials are used fresh or dried for vaccination of cattle under a wide range of conditions in the field. Ma & Fang (1949) concluded that the virus was not very resistant to temperature variation and apparently remained viable longer in blood and lymph nodes than in spleen; the diluent used was glycerol and buffered saline in equal quantities. Infected defibrinated blood was considered to be the best diluent in which to suspend material for desiccation, and a 10% dilution remained viable for 104 hours at 22° C. The storage time limits for these blood and tissue concentrates as used for vaccination where facilities for freeze-drying are not available require very thorough investigation, for success depends entirely upon transport of viable vaccine to the site of operations and the viability may well vary within very fine limits of time and temperature. Most workers have indicated that, on ice, tissue concentrates of 10 or 20% in blood can be used up to 48 hours; in East Africa 25% concentrate was stored at between 0° and +5° C. and the virus content titrated at intervals in rabbits; the smallest loss recorded in 24 hours was 2 $\log_{10}$ units or a drop in titre of one hundredfold. This was repeated many times with similar results and although the cause was never determined and several factors might have been involved, e.g. variation in temperature between 0° and 5°, contamination, or enzyme action, the danger of using a vaccine of this nature was obvious. Scott (1954b) noted that the addition of equal quantities of fresh bile to fluid vaccine killed the virus.

Peng et al. (1948) found freeze-dried vaccine viable for 105 days at +4° C. and Piercy (1953) confirmed its viability under vacuum at a similar temperature for three months. More complete figures on storage limits for freeze-dried and reconstituted vaccines were determined by Scott & Brotherston (1952), while Purchase et al. (1953) found vaccine fully potent after fourteen and a half months under vacuum at −20° C. The effect of residual moisture content upon the viability of stored virus is unknown, but Scott & MacLeod (1955a) found that ampoules deliberately broken to release vacuum were identical in titre up to 40 days at −20° C., but not after 112 days. Experimental work in East Africa indicated that lapinized vaccine reconstituted for use in the field must be used within 30 min. and any residue discarded after that time.

Immunity

According to Nakamura et al. (1938), rabbits injected with lapinized virus or with bovine virus resisted challenge with lapinized virus. Walker (1947) found that an avianized substrain protected rabbits against the parent lapinized virus. In East Africa, bovine virus has not been shown to protect rabbits and it has been difficult to obtain enough surviving rabbits to challenge the immunity; in one unconfirmed experiment, a single rabbit was re-infected with lapinized virus, after coming through a typical reaction three months previously, and died with typical lesions of rinderpest. Neutralizing antibodies and virus occur in rabbits simultaneously up to 14 days after infection and as neutralizing antibodies persist for at least four months, but perhaps not indefinitely, it is conceivable that rabbits become susceptible again after resolution of the lesions.

Cattle were shown to be immunized by lapinized virus, either alone or with immune serum (Edwards, 1927; Nakamura et al., 1943; Isogai, 1944; Cheng & Fischman, 1949) and workers in many countries have reported successful vaccination of different breeds; a visible thermal or other reaction is apparently unnecessary in some individuals for immunity to be effected. Brotherston (1951a, c) demonstrated an interference phenomenon, provided that the virus challenge was delayed for 84 to 108 hours after the injection of the vaccine and showed that this limited the spread of an epidemic in the field. These observations were confirmed by Illartein & Guerret (1954) and by Simpson (1954).

Buffaloes are immunized with the vaccine (Haddow & Idnani, 1947; Iyer & Srinivasan, 1954; Stevenin et al., 1955), while goats and sheep are vaccinated as a routine in India (Datta et al., 1951).

Experiments on duration of immunity have not been carried out under controlled conditions but under field conditions where reinforcement of immunity by contact could be reasonably excluded the duration has been 12 months (Nakamura et al., 1943), 19 months (Piercy, 1953) and 41 months (Datta, 1954). One feels that duration of immunity might well be related to the degree of reaction and therefore to the susceptibility of the animal, those reacting mildly having the shortest duration. Instances have occurred in which duration has varied in individuals of the same age and breed according to the method of challenge; Brotherston (1951b) reported that it could be 8–15 months with parenteral challenge and 13 months with challenge by contact with cattle reacting to field virus.

The most suitable age for vaccination has not been investigated.

The effect of the virus upon animals with an acquired resistance to rinderpest requires to be studied, for Brotherston & Purchase (1952) found that cattle previously vaccinated with inactivated spleen tissue could be re-immunized 14 months later with lapinized virus, while Piercy (1953) showed that it was not effective in cattle given inactivated vaccine 9 months before. The question of re-immunizing animals with lapinized or goat-adapted virus has not been studied and is a subject of much importance in the control of rinderpest.

Serology

Neutralizing antibodies were developed in the rabbit five days after inoculation although the virus persisted in the blood for nine days longer (Nakamura, 1940) and these antibodies could be detected for at least four months. Complement-fixing antibodies appeared at eight days and persisted for one month although the titre dropped (Nakamura & Goto, 1941).

Immune bovine serum neutralized lapinized virus (Baker, 1946; Jenkins & Walker, 1946). Japanese black cattle vaccinated with lapinized virus developed complement-fixing and neutralizing antibodies, but the latter were a more sensitive and durable indicator of immunity according to Fukusho et al. (1953) and this was confirmed by Nakamura et al. (1955) in Korean cattle. A neutralization test using chick embryos was studied by Furutani et al. (1954), who found that the intravenous route gave better results than the yolk sac. Both types of antibody have been demonstrated in pigs injected with lapinized virus and an avian substrain by Nakamura et al. (1954), but the animals gave no physical indication of infection; by this means it may therefore be possible to demonstrate an increase in immunity in animals which apparently fail to react to the vaccine because of an acquired resistance. There is no doubt that the relationship between strains of lapinized virus can be investigated and the immune reaction studied more fully by these means. Brown (1956) detected an early immune response in very young calves to the injection of lapinized virus.

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